TAIBAH UNIVERSITY

COLLEGE OF PHARMACY

Department of Pharmaceutics&

Pharmaceutical Technology

Handbook on Practical
Pharmaceutical Microbiology II

Contents

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2- Diagnosis of microbial infection of Gram positive bacteria.
3- Diagnosis of microbial infection of Gram negative bacteria.
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13- Protozoa: Ciliphora and Coccidia.

Module 1

SAFETY MEASUREMENT

Universal Precautions for Microbiology Laboratories

Blood and other body fluids' from all patients should be considered infective.

1. All specimens of blood-and body fluids should be put in a well constructed container with a secure lid to prevent leaking during transport. Care should be taken when collecting each specimen to avoid contaminating the outside of the container and' of the laboratory; form accompanying the specimen.

2. All persons processing blood and body-fluid specimens should wear gloves. Masks and protective eyewear should be worn if contact of mucous membrane with blood or body fluids is anticipated. Gloves should be changed and hands should be washed after completion of specimen processing.

3. For routine procedures, such, as histological and pathological studies or microbiologic culturing, a biological safety cabinet is not necessary. However, biological safety cabinets should be used whenever procedures are conducted that have a high potential for generating droplets: These include activities such as blending, sonicating and vigorous mixing.

4. Mechanical pipetting devices should; be used for manipulating all liquids in the laboratory. Mouth pipetting must not be done.

5. Use of needles and syringes should be limited to situations in which there is no alternative, and the recommendations for preventing injuries with needles outlined under universal precautions should be followed.

6. Laboratory work surfaces should be decontaminated with an appropriate chemical germicide after a spill of blood or other body fluids and when work activities are completed.

7. Contaminated materials used in laboratory tests should he decontaminated before reprocessing or be placed in bags and disposed of in accordance with institutional policies for disposal of in infective waste.

8. Scientific equipment that has been contaminated with blood or other body fluids should be decontaminated and cleaned before being repaired in the laboratory or transported to the manufacturer.

9. All persons should wash their hands after completing laboratory activities and should remove protective clothing before leaving the laboratory.

10. There should be no eating, drinking, or smoking in the work area.

Module 2

DIAGNOSIS OF MICROBIAL INFECTION OF GRAM POSITIVE BACTERIA.

Importance of bacterial identification

- Determining the appropriate antibacterial therapy.
- Determining either infectious organisms are risk for others patients in the hospital, the public and other laboratory workers.

Identification methods

- Traditional method/phenotypic method
- Immunochemical method/serological methods
- Genotypic method/molecular method

Phenotypic criteria

- Microscopic morphology and staining characteristics.
- Macroscopic (colony) morphology.
- Growth conditions
- Resistance or susceptibility to antimicrobial agent.
- Nutritional requirements and Metabolic pathways

a) Staining Reactions

- Gram stain divides bacteria into Gram positive and Gram negative
- Ziehl-Neelsen stain divides them into acid fast and non acid fast
- Special stains are necessary to bring out characteristics like capsules, spores.

b) Macroscopic morphology

- Study colonies on solid media:

- Size, Shape, Margins, Surface, elevations, Edge, colour, consistency, pigmentation, haemolysis.
-
- In fluid :
- Degree of growth – Absence, scanty, moderate, abundant etc.
- Presence of turbidity and its nature
- Presence of deposit and its character
- Nature of surface growth and odour

c) Environmental requirement for growth

- Four most critical factor
- Oxygen and CO2 availability
- Facultative anaerobic: most clinically significant aerobic organisms
- Strictly aerobic: pseudomonas.
- Anaerobic: Clostridium
- Capnophilic: Neisseria spp.
- Temperature: 35-37 0C
  - 42 0C : Compylobacter jejuni
- Neutral pH-range 6.5-7.5
- Moisture

d) Resistance or susceptibility to antimicrobial agent

- The ability of an organism to grow in the presence of certain antimicrobial agents is widely used to establish preliminary identification information

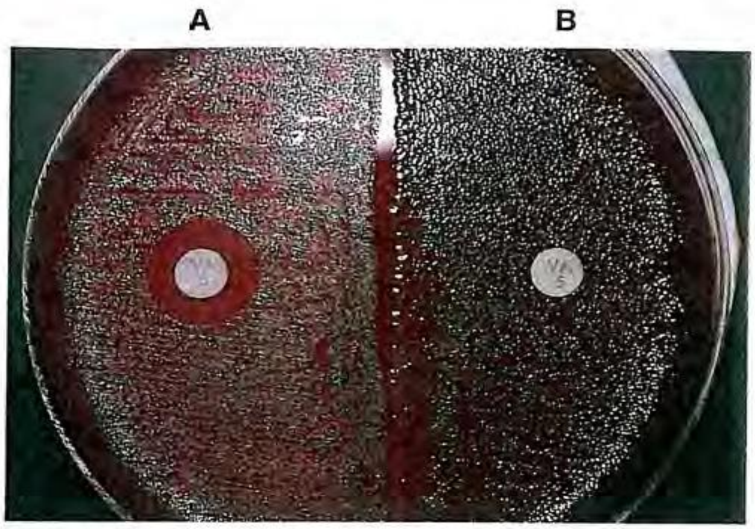
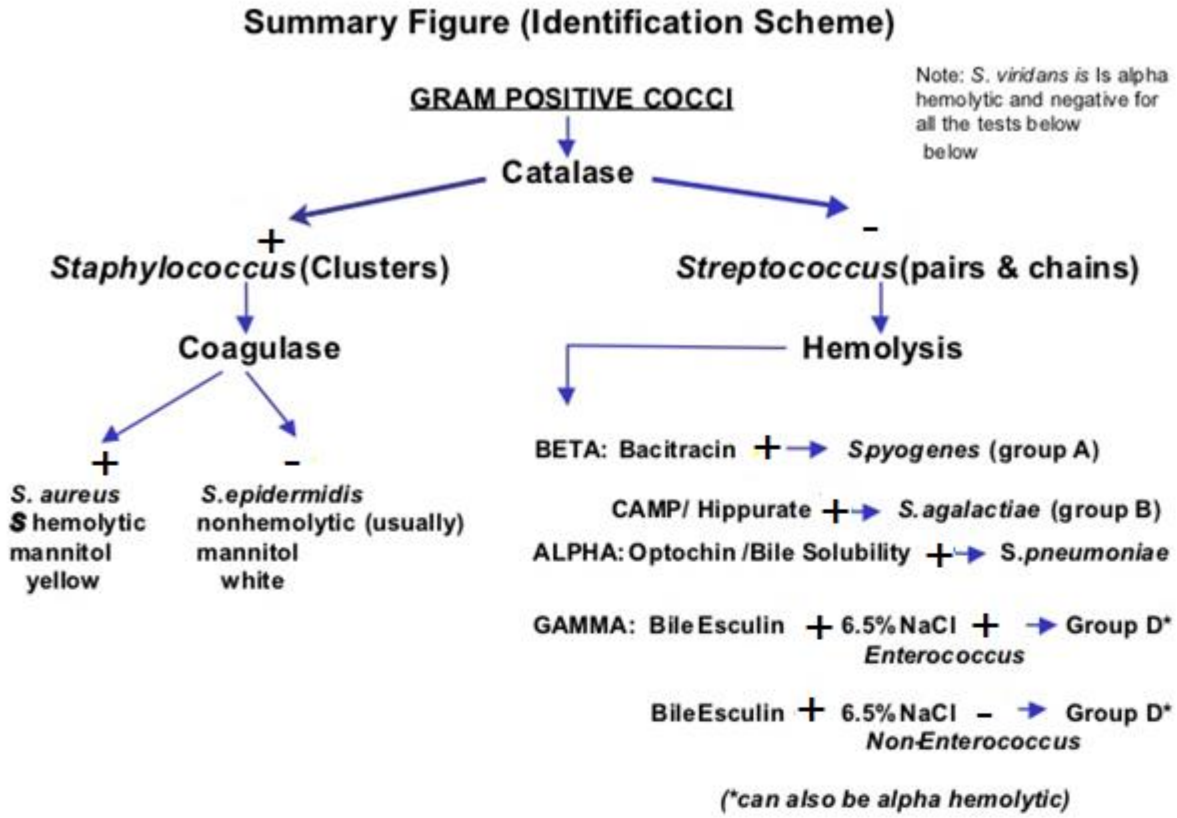


Figure 7-15 A, Zone of growth inhibition around the 5-mg vancomycin disk is indicative of a gram-positive bacterium. B, The gram-negative organism is not inhibited by this antibiotic, and growth extends to the edge of the disk.

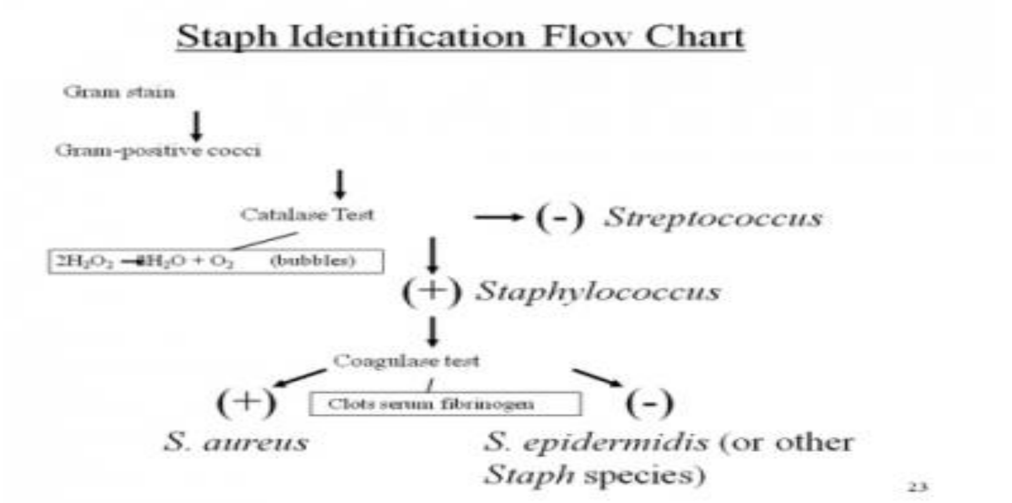
e) Nutritional requirements and Metabolic pathways

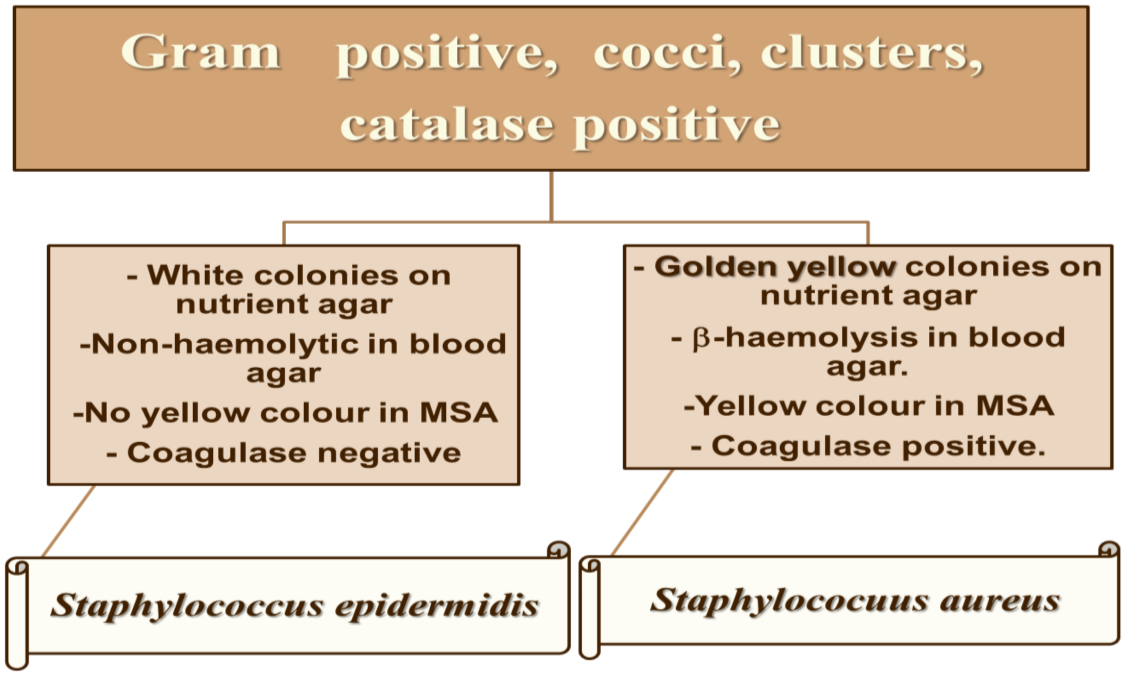
- It is the most common approach used for determining the genus and species of organism
- In general all methods available are a combination of tests.
- to establish the enzymatic capabilities of a given bacterial isolate.
- Isolates ability to grow or survive in the presence of certain inhibitors(e.g. salt, toxins, antibiotics)

A. Gram positive bacteria


Summary Figure (Identification Scheme)
GRAM POSITIVE COCCI
Note: S. viridans is Is alpha hemolytic and negative for all the tests below below
Catalase
+
Staphylococcus(Clusters)
-
Streptococcus(pairs & chains)
Coagulase
Hemolysis
+
S. aureus
S hemolytic
mannitol
yellow
-
S.epidermidis
nonhemolytic (usually)
mannitol
white
BETA: Bacitracin + Spyogenes (group A)
CAMP/ Hippurate + S.agalactiae (group B)
ALPHA: Optochin /Bile Solubility + S.pneumoniae
GAMMA: Bile Esculin + 6.5% NaCl + Group D* Enterococcus
Bile Esculin + 6.5% NaCl - Group D* Non-Enterococcus
(*can also be alpha hemolytic)

I-*Staphylococcus spp.*




Gram positive, cocci, clusters, catalase positive
- White colonies on nutrient agar
-Non-haemolytic in blood agar
-No yellow colour in MSA
- Coagulase negative
- Golden yellow colonies on nutrient agar
- β-haemolysis in blood agar.
-Yellow colour in MSA
- Coagulase positive.
Staphylococcus epidermidis
Staphylococuus aureus

1- ***Staphylococcus aureus***

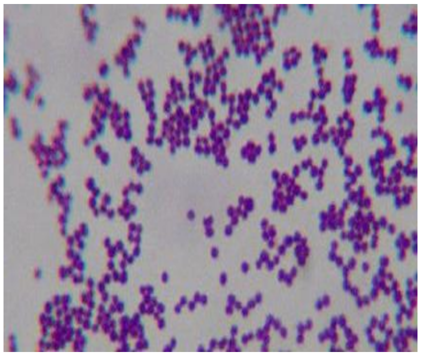
Staphylococcus aureus is a Gram-positive coccal bacterium and is frequently found in the nose, respiratory tract, and on the skin. Term "staphylococcus" is derived from the Greek (staphyle: mean bunches of grapes and kokkos= berry); meaning that bacteria occurring in grape like cluster or beery.

Although *S. aureus* is not always pathogenic, it is a common cause of skin infections such as abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing potent protein toxins, and expressing cell-surface proteins that bind and inactivate antibodies. The emergence of antibiotic-resistant strains of *S. aureus* such as methicillin-resistant *S. aureus* (MRSA) is a worldwide problem in clinical medicine.

Laboratory identification

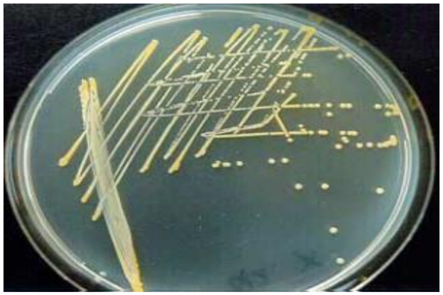
a- Gram stain

Gram positive cocci, Grape like arrangement



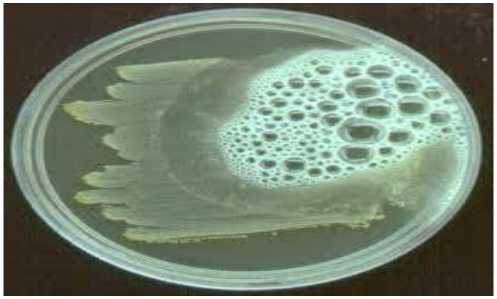
b- Nutrient Agar

Golden yellow colonies is observed when *S. aureus* was grown on nutrient agar



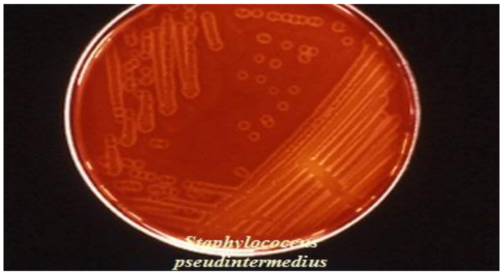
c-Catalase test

- The catalase production by organism is indicated by effervescence in presence of H_2O_2
- Catalase positive in all Staphylococcus species



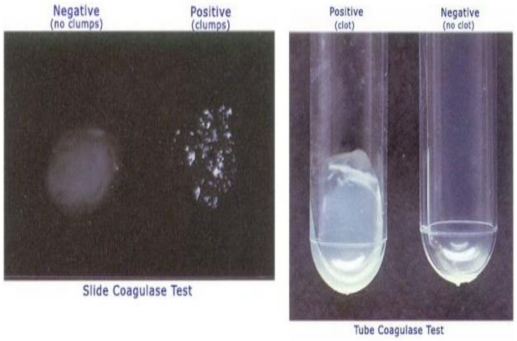
d- Blood agar

- Complete haemolysis with clear zone around the colonies (β- haemolysis)



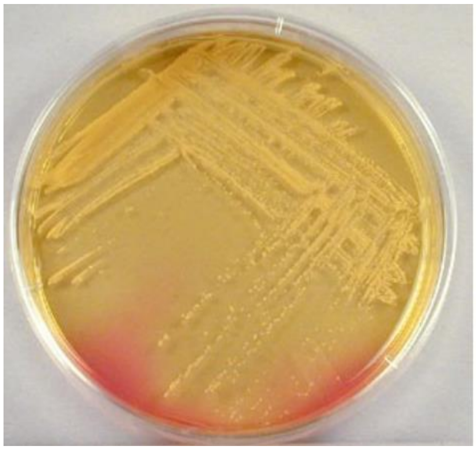
e- Coagulase test

- Coagulase enzyme is produced by *S. aureus*
- No Coagulase production in other Staphylococci spp.
- The enzyme converts fibrinogen to fibrin by reacting with prothrombin forming a complex known as staphylothrombin



f- Mannitol Salt Agar (MSA)

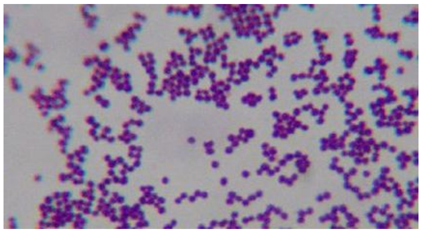
- The high concentration of sodium chloride (7.5% ~ 10%) inhibits most other bacteria in specimen.
- *S. aureus* produces yellow colonies with yellow zones due to its ability to ferment mannitol and produce acids which convert phenol red indicator to yellow



2- *Staphylococcus epidermidis*

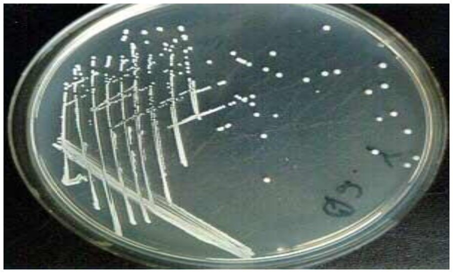
b- Gram stain

Gram positive cocci, Grape like arrangement



b- Nutrient Agar

White colonies on nutrient agar

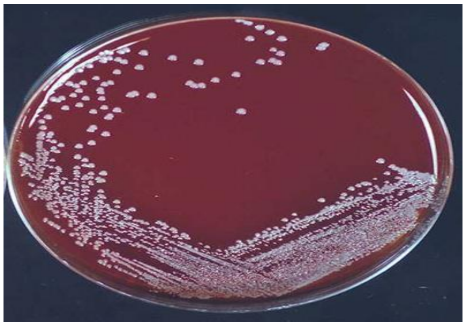


c-Catalase test

- The catalase production by organism is indicated by effervescence in presence of H2O2
- Catalase positive in all Staphylococcus species

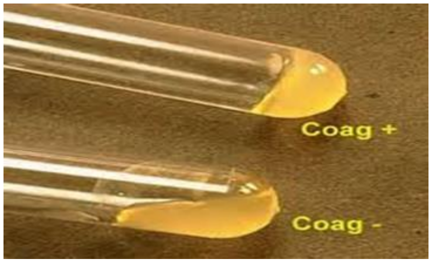
d- Blood agar

Non-haemolytic in blood agar



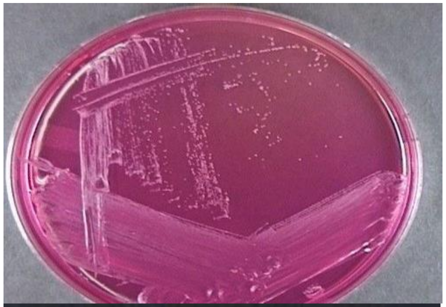
e- Coagulase test

- **Coagulase negative**

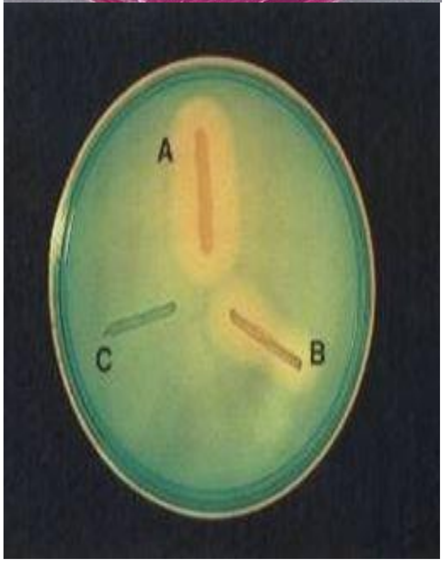


f- Mannitol Salt Agar (MSA)

- **No yellow colour in MSA**



g- DNAse test

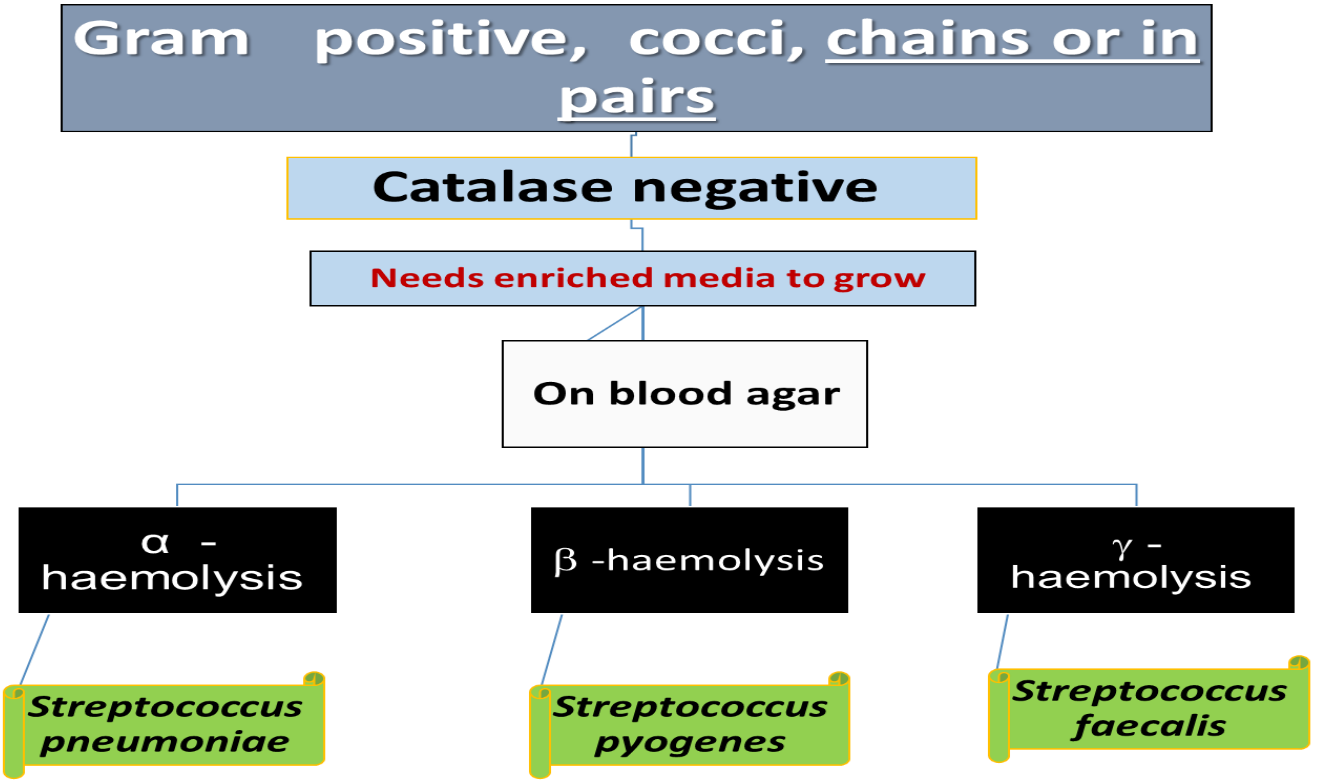


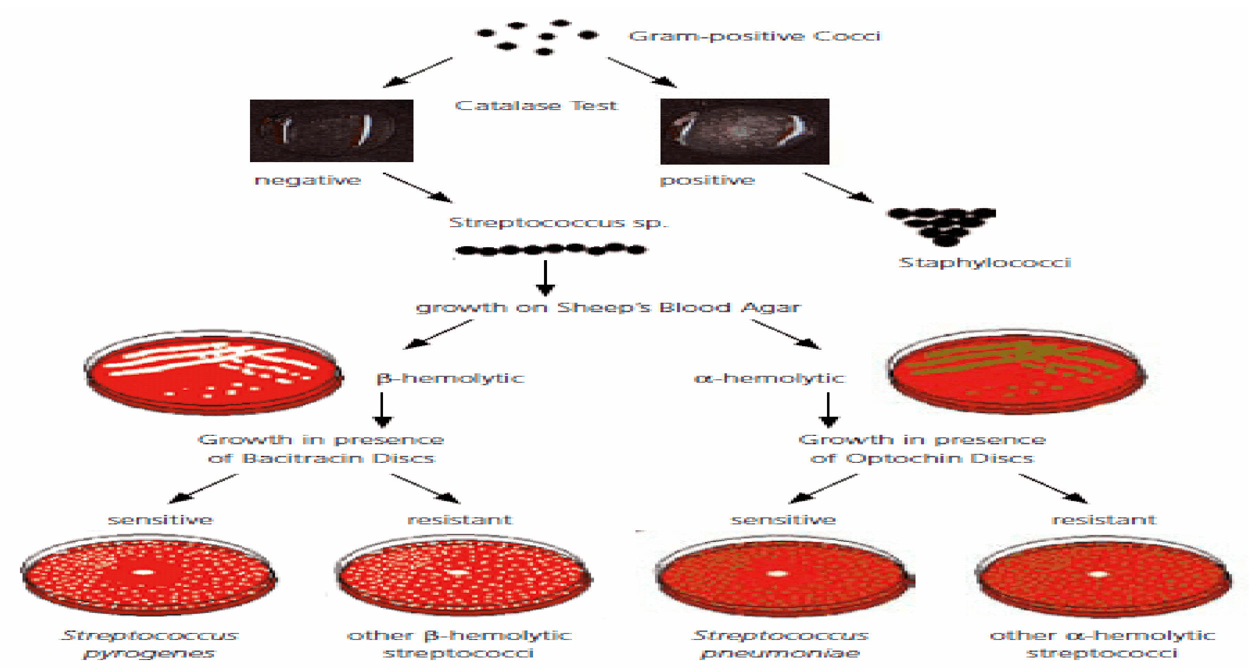
DNA hydrolysis test or Deoxyribonuclease (DNase) test is used to determine the ability of an organism to hydrolyze DNA and utilize it as a source of carbon and energy for growth.

This medium is **pale green in color** because of DNA-methyl green (indicator) complex (Note: Methyl green is a cation which binds to the negatively-charged DNA). It also contains nutrients for the bacteria.

Positive: When DNA is hydrolyzed, methyl green is released turning the medium colorless around the test organism.

II- Streptococus spp.





Streptococcus agalactiae **Streptococcus Viridans**

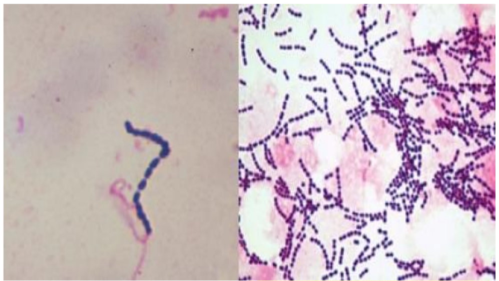
Streptococci are facultatively anaerobic, Gram-positive organisms that often occur as chains or pairs. Streptococci are subdivided into groups by antibodies that recognize surface antigens (figure 4). These groups may include one or more species. The most important groupable streptococci are A, B and D. Among the groupable streptococci, infectious disease (particularly pharyngitis) is caused by group A which is thus emphasized here. *Streptococcus pneumoniae* (a major cause of human pneumonia) and *Streptococcus mutans* and other so-called viridans streptococci (among the causes of dental caries) do not possess group antigens.

1- ***Streptococcus pyogenes***

Laboratory Identification

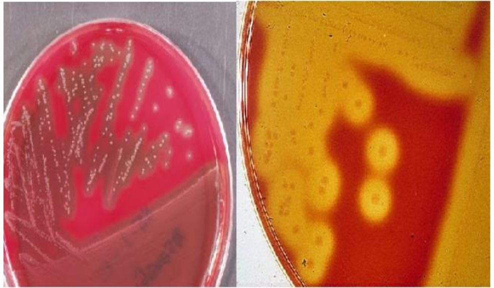
a- Gram stain

Streptococcus: Cocci in chains



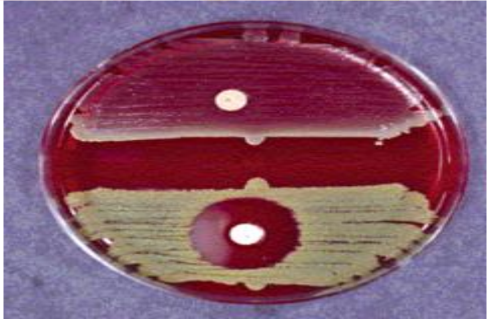
b- On Blood agar:

Complete haemolysis.



c- Bacitracin sensitivity test:

Streptococcus pyogenes (group A streptococci) is inhibited by the small amount of bacitracin in the disk.



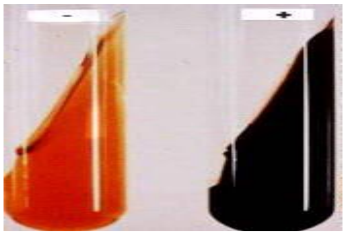
d- Pyrrolidonyl aminopeptidase test

The substrate for the PYR test is L-naphthylamide-β-naphthylamide which is hydrolyzed by a specific bacterial aminopeptidase enzyme.

Hydrolysis of the substrate by pyrrolidonyl aminopeptidase enzyme releases free β-naphthylamide, which is detected by the addition of N, N-dimethylaminocinnamaldehyde. This detection reagent couples with the naphthylamide to form a red Schiff base.

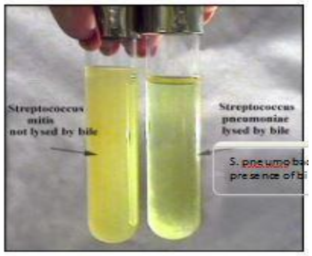
2-*Streptococcus pneumonia*

a- Bile Esculin test: negative



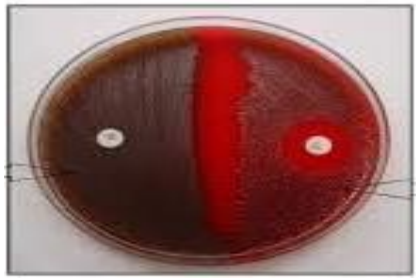
medium contains bile and esculin, in presence of enterococcus esculin turned to esculitin and dextrose. Esculitin with ferric chloride (present in medium) gives black ppt

b- Bile solubility test: positive



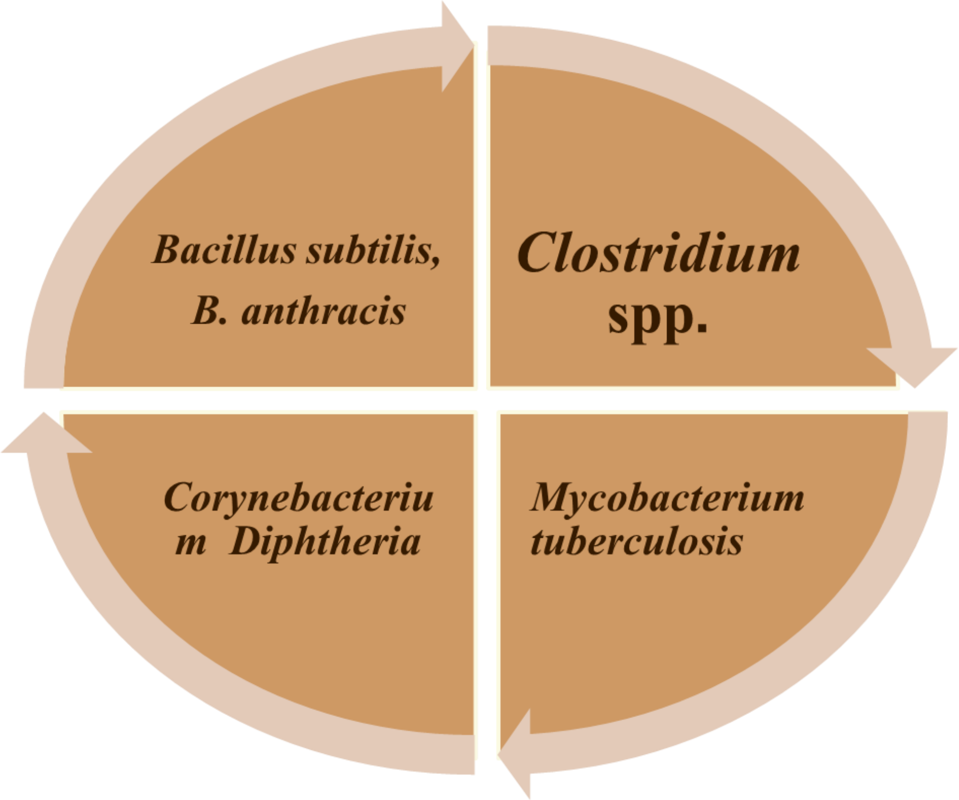
bile salts as sod deoxcholate + suspension of *S. pneumoniae* in saline -------→ observe clear solution during 3 hrs due to cell wall of strept lysis by bile salts

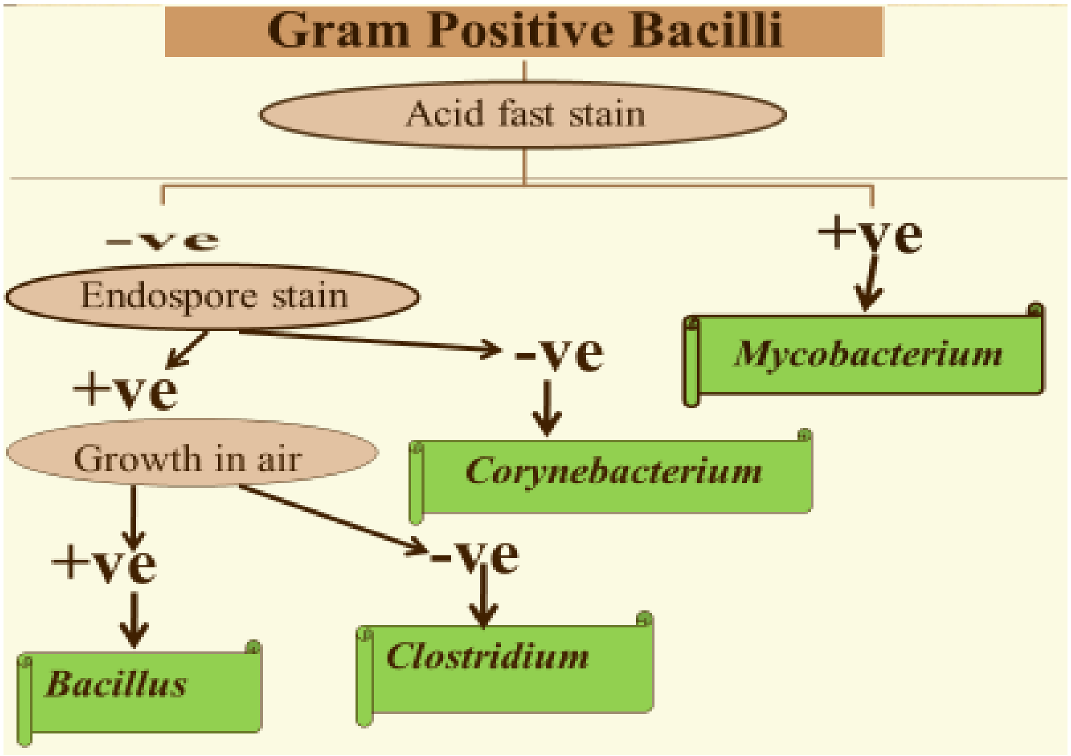
c- Optochin sensitivity test: sensitive



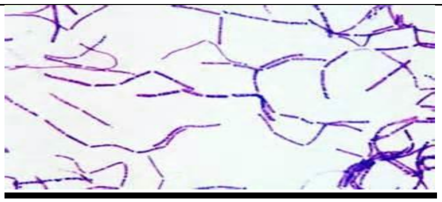
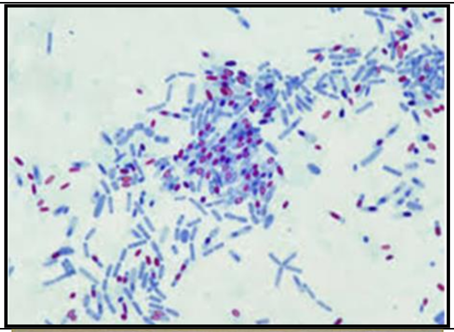
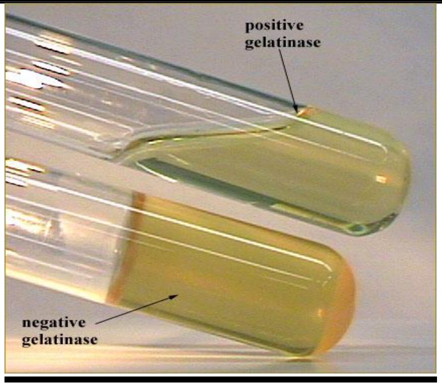
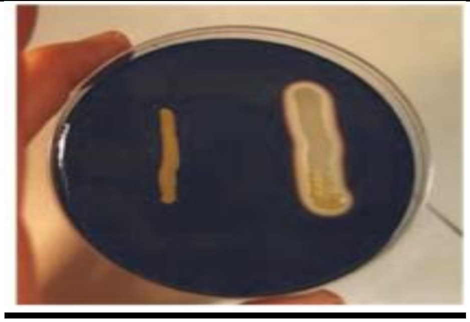
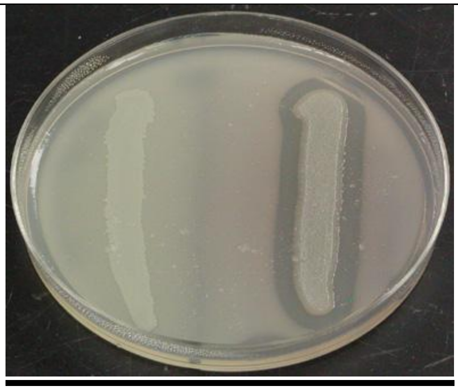
The optochin (ethylhydrocupreine hydrochloride) test is a presumptive test that is used to identify strains of *Streptococcus pneumoniae*. Optochin disks are placed on inoculated blood agar plates. Because *S. pneumoniae* is not optochin resistant, a zone of inhibition will develop around the disk where the bacteria have been lysed. This zone is typically 14mm from the disk or greater.

Gram Positive Bacilli





Bacillus

a. Gram stain Gram positive bacteria	
b. Spore Stain (Modified acid fast stain) the acid-fast cells will appear red to pink in color, whereas the non-acid-fast cells will appear blue.	
c. Gelatinase test The medium is solidify by adding gelatin instead of agar. The organism is inoculated by stabbing After incubation, positive result is obtained when organism liquify gelatin.	
d. Amylase test When Bacillus grows in medium containing starch, amylase enzyme elaborates and hydrolyse starch. No blue colour with iodine is observed around the colonies.	
e. Caseinase test When Bacillus grows in medium containing casein, caseinase enzyme elaborates and hydrolyses casein to polypeptide. No precipitation is observed around colonies on adding HCl.	

Laboratory Report 1

1- Gram stain:………………………………

2- Shape:…………………………………….

3- Arrangement:……………………………

4- Color:…………………………………….

5- Confirmatory test:

NO:	Name	Result
1		
2		
3		
4		

6- Drawing:

7-Conclusion **The organism is:**

Laboratory Report 2

1- Gram stain:………………………………

2- Shape:…………………………………….

3- Arrangement:……………………………

4- Color:…………………………………….

5- Confirmatory test:

NO:	Name	Result
1		
2		
3		
4		

6- Drawing:

7-Conclusion **The organism is:**

Laboratory Report 3

1- Gram stain:………………………………

2- Shape:…………………………………….

3- Arrangement:……………………………

4- Color:…………………………………….

5- Confirmatory test:

NO:	Name	Result
1		
2		
3		
4		

6- Drawing:

7-Conclusion **The organism is:**

Laboratory Report 4

1- Gram stain:………………………………

2- Shape:…………………………………….

3- Arrangement:……………………………

4- Color:…………………………………….

5- Confirmatory test:

NO:	Name	Result
1		
2		
3		
4		

6- Drawing:

7-Conclusion **The organism is:**

Laboratory Report 5

1- Gram stain:………………………………

2- Shape:…………………………………….

3- Arrangement:……………………………

4- Color:…………………………………….

5- Confirmatory test:

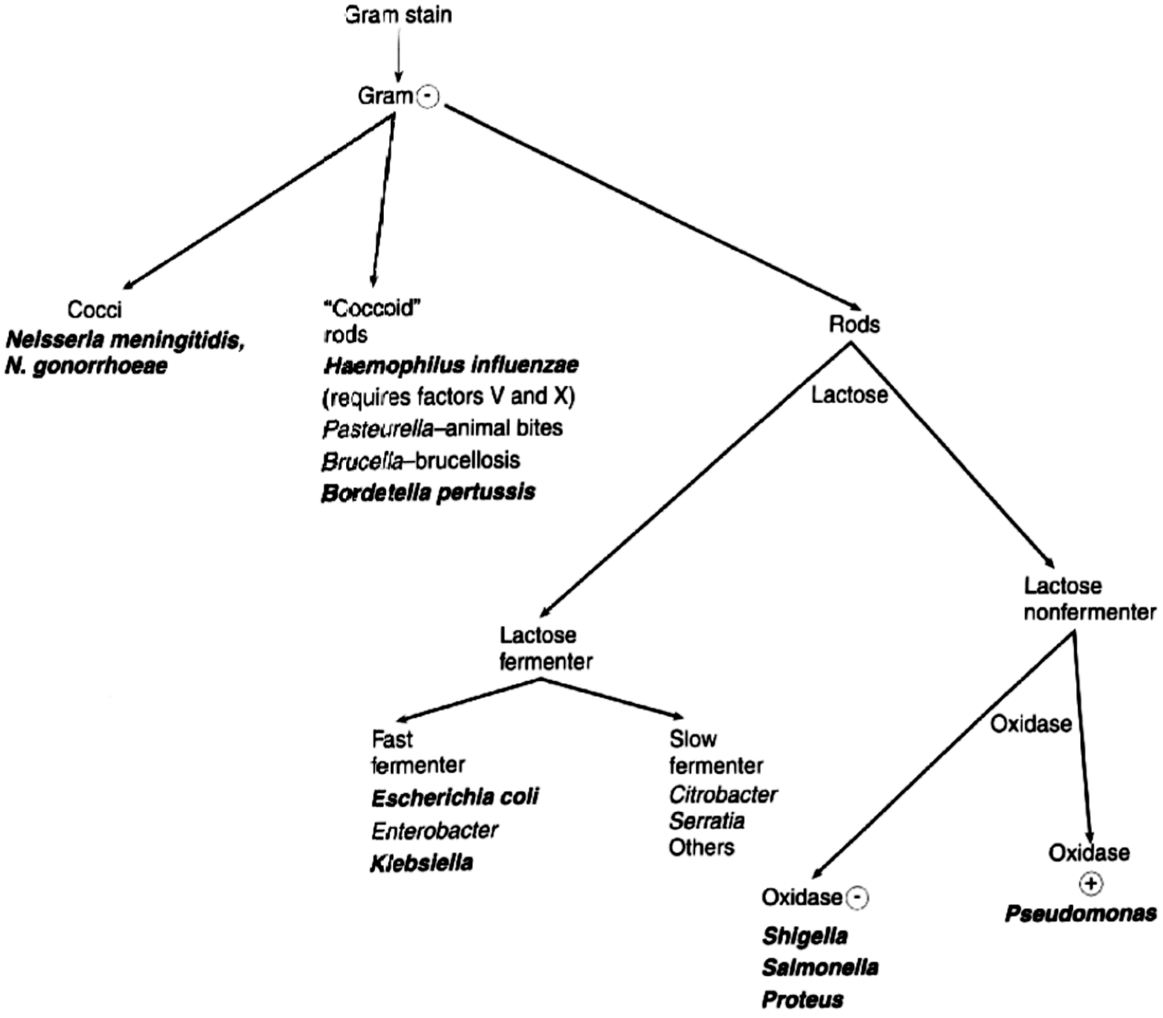
NO:	Name	Result
1		
2		
3		
4		

6- Drawing:

7-Conclusion **The organism is:**

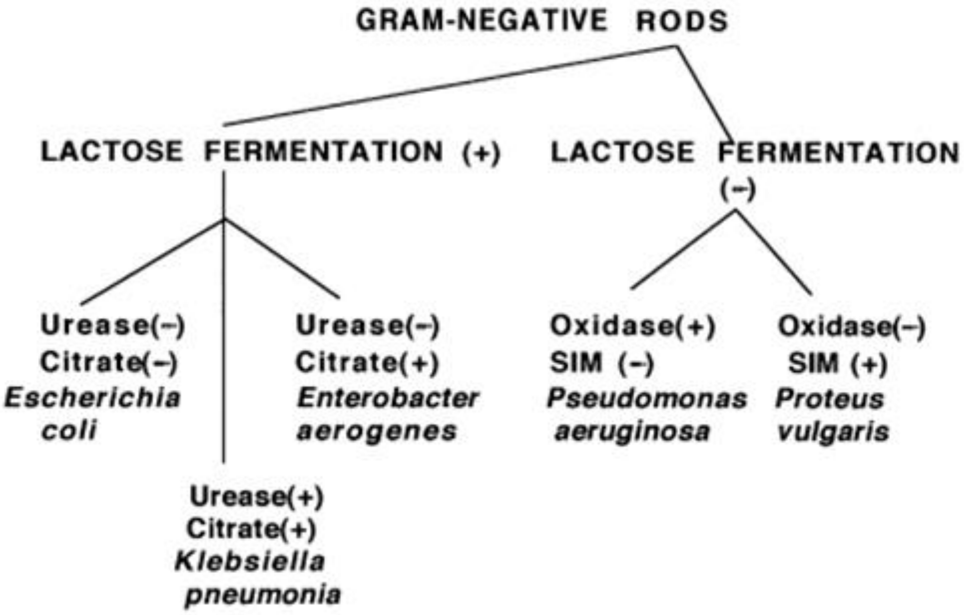
Module 3

DIAGNOSIS OF MICROBIAL INFECTION OF GRAM NEGATIVE BACTERIA.



I-The Family Enterobacteriaceae

The Enterobacteriaceae are a family of Gram-negative , facultatively anaerobic , rod shaped bacteria , usually motile , made up of saprophytes and plant and animal parasites of worldwide distribution, found in soil, water, and plants and in animals from insects to humans. In humans, disease is produced by both invasive action and production of toxin. Species not normally associated with disease are often opportunistic pathogens. Enterobacteriaceae have been responsible for as many as half of the nosocomial infections reported annually in the United States, most frequently by species of Escherichia, Klebsiella, Enterobacter,Proteus, Providencia, and Serratia.

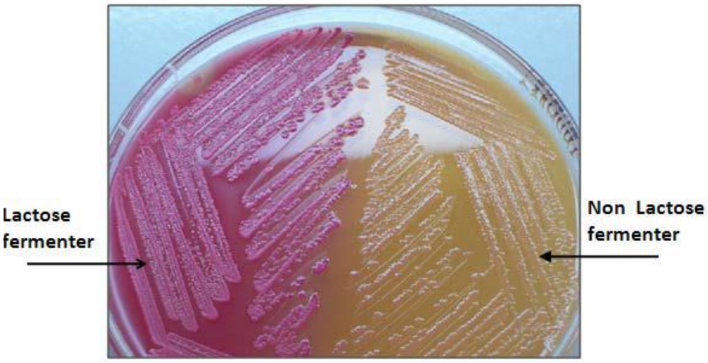


Common characteristics of family Enterobacteriaceae are:

1- They are Gram negative, short rods.

2- They are non-sporulating, **facultative anaerobes**

3- They have simple nutritional requirements and **MacConkey agar** is used to isolate and differentiate organisms of Enterobacteriaceae family *(Pink colored colonies of lactose fermenter-coliforms and pale colored colonies of Non lactose fermenter)*



- ☐ **Lactose fermenters:**
  (CEEK)
  1- *Citrobacter*
  2- *Escherichia*
  3- *Enterobacter*
  4- *Klebsiella*
- ☐ **Non lactose fermenter**
  (ShYPS)
  1- *Shigella*
  2- *Yersinia*
  3- *Proteus*
  4- *Salmonella*

Tests for identification of members of Enterobacteriaceae family

Member of the Enterobacteriaceae family are identified based on their biochemical properties. Commonly used biochemical tests to identify them are:

1- Citrate utilization Test
2- Indole Test
3- Motility Test
4- Methyl Red (MR) Test
5- Voges–Proskauer (VP) Test
6- Triple Sugar Iron (TSI) Agar
7- Urease Test

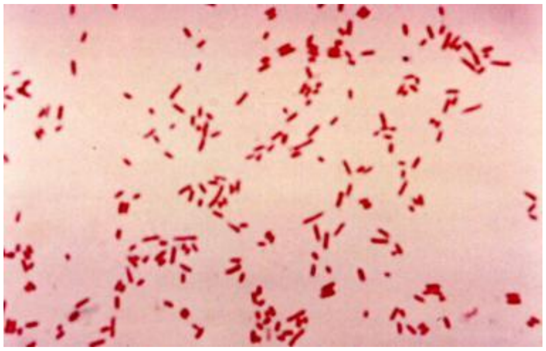
Escherichia coli

Escherichia coli (E. coli) is a bacterium that is commonly found in the gut of humans and warm-blooded animals.

Most *E. coli* are normal commensals found in the intestinal tract. It is a Gram negative short rod, member of the family Enterobacteriaceae. Pathogenic strains of *E. coli* are distinguished from normal flora by their possession of virulence factors such as exotoxins.

Laboratory identification

1-Gram stain



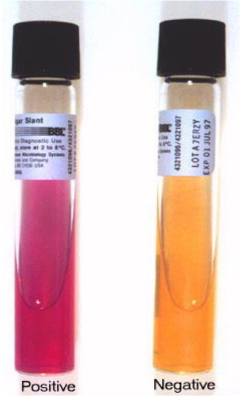
E. coli is a Gram negative organism

2- MacConkey's Medium

Test the ability of the organism to ferment lactose and produce acid that convert the color of neutral red into purple. *E. coli* is lactose fermenter so it turns the color of the colonies into pink color.

3- Urease production test:

- To test the ability of the organism to produce urease enzyme which split urea in urea media to form ammonia and CO2.
- The accumulation of the ammonia will produce alkaline PH which turns the color of the indicator (phenol red) into pink.
- *E. coli* is negative urease test



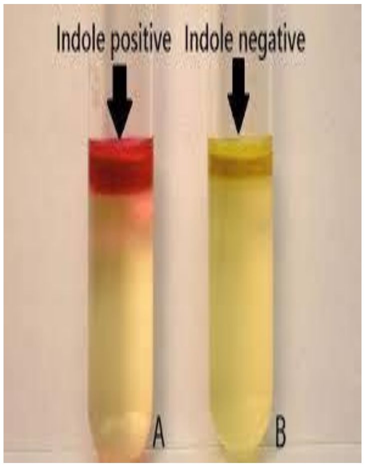
4- IMViC

IMViC is abbreviation to the following tests

✓ **Indole**
✓ **Methyl red**
✓ **Voges-Proskauer**
✓ **Citrate**

A- Indole test:

Test for the ability of the microorganism to break down tryptophan into indole.



Procedure:

☐ Incubate tryptone broth media with the tested organism.

☐ The presence of indole can be detected through the use of Kovac’s reagent (para dimethy aminobenzaldehyde in amyl alcohol).

☐ Kovac's reagent, which is yellow, reacts with indole and produces a red color on the surface of the test tube.

☐ *E. coli* is positive with indole test.

B- Methyl red test:

☐ In methyl red test the test organism can ferment the glucose and produce acid which will change the color of the Methyl red indicator into red color.

- *E. coli* is one of the bacteria that produce acids, causing the pH to drop below 4.4. The pH indicator methyl red will turn red (a positive MR test).
- *Klebsiella* and *Enterobacter* produce neutral products from glucose (e.g. Acetoin) causing the pH to rise above 6.2. At this pH, methyl red indicator is a yellow (a negative MR test).

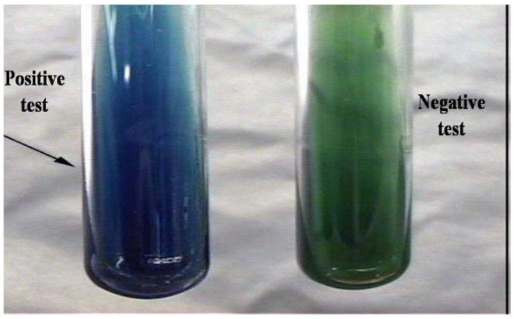
C- Vogues-Proskaure’s test

- The reagents used for the VP test are Barritt's A (alpha-napthol) and Barritt's B (potassium hydroxide).
- When these reagents are added to a broth in which acetoin is present; they produce a red color (a positive VP test). This color may take 20 to 30 minutes to develop.
- *E. coli* does not produce acetoin, but *Enterobacter* and *Klebsiella* do.

Methyl Red-Voges Proskauer Tests

- ***E. coli* is (MR+/VP-).**
- ***Enterobacter* and *Klebsiella* are (MR-/VP+).**

D-Citrate utilization test:



- The citrate test is used to determine the ability of a bacterium to utilize citrate as the only source of carbon.
- Bacteria can break citrate into organic acids and carbon dioxide.
- The carbon dioxide can form a basic compound; sodium carbonate.

- A pH indicator Bromothymol Blue in the medium detects the presence of this compound by turning blue (a positive test).
- *E. coli* is negative with citrate test.

5- Triple Sugar Iron Agar (TSI)

Whenever you see the name of this test i.e. Triple Sugar Iron Agar ,you have to remember that it’s a test which has **three sugar** (Lactose, Sucrose, and Glucose) and also iron; and it contains Agar Agar as solidifying agent (TSI is a semi solid media having slant and butt).

Composition of Triple Sugar Iron Agar (TSI)

- **0.1% Glucose**: If only glucose is fermented, only enough acid is produced to turn the butt yellow. The slant will remain red
- **1.0 % lactose/1.0% sucrose:** a large amount of acid turns both butt and slant yellow, thus indicating the ability of the culture to ferment either lactose or sucrose.
- **Iron:** Ferrous sulfate: Indicator of H_2S formation
- **Phenol red:** Indicator of acidification (It is **yellow in acidic condition** and red under alkaline conditions).
- It also contains **Peptone** which acts as source of nitrogen. (Remember that whenever peptone is utilized under aerobic condition ammonia is produced)

Interpretation of Triple Sugar Iron Agar Test

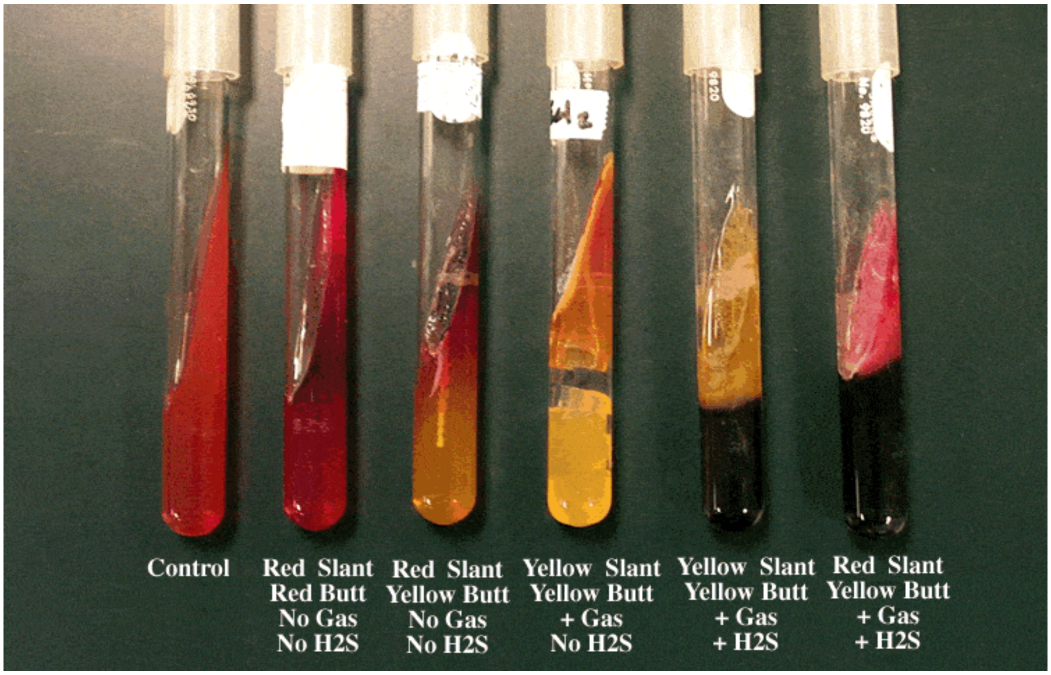
1- If lactose (or sucrose) is fermented, a large amount of acid is produced, which turns the phenol red indicator yellow both in butt and in the slant. Some organisms generate **gases**, which produces **bubbles/cracks** on the medium.

2- If **lactose is not fermented** but the small amount of glucose is, the oxygen deficient **butt will be yellow** (remember that butt comparatively have more glucose compared to slant i.e. more media more glucose), but on the slant the acid (less acid as media in slant is very less) will be oxidized to carbon dioxide and water by the organism and the **slant will be red** (alkaline or neutral pH).

3- If **neither lactose/sucrose nor glucose is fermented, both the butt and the slant will be red**. The slant can become a deeper red-purple (more alkaline) as a result of production of ammonia from the oxidative deamination of amino acids (remember peptone is a major constituents of TSI Agar).

4- If H_2S is produced, the black color of ferrous sulfide is seen.

So the expected results of TSI Agar test are:



Triple Sugar Iron Agar Test Results

1- Alkaline slant/no change in butt (K/NC) i.e Red/Red = glucose, lactose and sucrose non-fermenter

2-Alkaline slant/Alkaline butt (K/K) i.e Red/Red = glucose, lactose and sucrose non-fermenter

3-Alkaline slant/acidic butt (K/A); Red/Yellow = glucose fermentation only, gas (+ or -), H2s (+ or -)

4-Acidic slant/acidic butt (A/A); Yellow/Yellow = glucose, lactose and/or sucrose fermenter gas (+ or -), H2s (+ or -).

Name of the organisms	**Slant**	**Butt**	**Gas**	**H2S**
Escherichia	Acid (A)	Acid (A)	Pos (+)	Neg (-)

6-Oxidase production

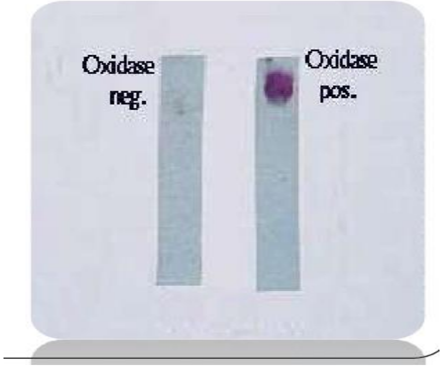
- It indicates the presence of cytochrome C in the respiratory chain.
- Aerobic microorganisms with cytochrome C can oxidize certain amines to form colored products.

MATERIALS:

- Filter paper 1% N,N,N'N' tetramethyl-P-phenylenediamine

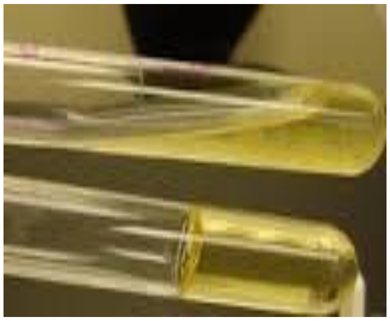
METHOD:

- Pre-wet filter paper with oxidase reagent (N,N,N',N'-P-phenylenediamine) and allow to dry.
- Pick a bacterial colony with a sterile toothpick.
- Gently scratch the colony onto the filter paper. A purple color is produced.



- ✓ ***E. coli* is oxidase negative**

7- Gelatin hydrolysis



- Objective: to test the ability of the microorganism to produce exoenzyme gelatinsae which liquefy gelatin.

  The gelatin hydrolysis is indicated by loss in ability to solidify even after refrigeration.

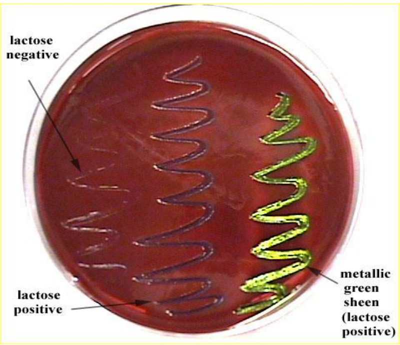
- *E. coli* gives negative result.

8-Catalase test:

- E.coli is catalase positive

9-Eosin Methylene Blue (EMB): differential medium

- Lactose, sucrose, eosin, and methylene blue and bile salts (to inhibit gm +ve bacteria)
- Strong fermentation of lactose give acid facilitate dye diffusion and give metalic sheen (E. coli)
- Weaker fermentation of lactose results in colonies with a pinkish-purple color. Citrobacter and Enterobacter
- Colonies of nonlactose fermenters remain colorless



Klebsiella pneumoniae

Klebsiella pneumoniae is a Gram-negative, nonmotile, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. Klebsiella appears as a mucoid lactose fermenter on MacConkey agar. Although found in the normal flora of the mouth, skin, and intestines, it can cause destructive changes to human and animal lungs if aspirated (inhaled), specifically to the alveoli (in the lungs) resulting in bloody sputum.

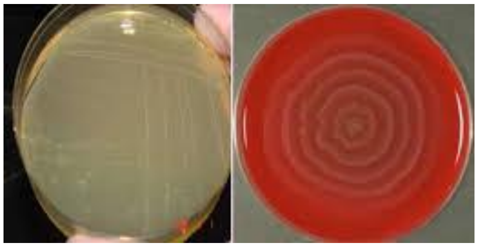
Laboratory identification

Test	**Result**
1-Gram Stain:	Gram negative organism
2-McConkey's Medium	lactose fermenter so it turn the color of the colonies into pink color.
3-Urease production test	positive urease test.
4-IMViC: **Indole test:** **Methyl red test:** **Vogues-Proskaure's test:** **Citrate utilization test:**	negative with Indole test. methyl red indicator is a yellow (a negative MR test). **Methyl Red-Voges Proskauer Tests** *Enterobacter* and *Klebsiella* are (MR-/VP+). positive with citrate test.
5- Triple Sugar Iron Agar (TSI)	Acidic slant and butt, gas(+), H2S(-)
6- Oxidase test:	*Klebsiella pneumonia* is oxidase negative
7-Motility agar:	*K. pneumonia* is non motile.
8-Gelatin hydrolysis:	*K. pneumonia is negative*

9- Catalase test:	*K. pneumonia is positive catalase test*

Proteus species

Proteus is a genus of Gram-negative Proteobacteria. Proteus bacilli are widely distributed in nature as saprophytes, being found in decomposing animal matter, sewage, manure soil, and human and animal feces. They are opportunistic pathogens, commonly responsible for urinary and septic infections, often nosocomial.

Test	**Result**
1-Gram Stain:	Gram negative organism
2-McConkey's Medium	Non lactose fermenter
3-Urease production test	positive urease test.
4-IMViC: **Indole test:** **Methyl red test:** **Vogues-Proskaure's test:** **Citrate utilization test:**	*Proteus vulgaris* is positive with Indole test while *Proteus mirabilis* is negative with Indole test. *Proteus* species **is (MR+/VP-)** *Proteus mirabilis* is positive with citrate test while *Proteus vulgaris* is negative.
5- Triple Sugar Iron Agar (TSI)	alkaline slant ,acid butt, gas(+), H2S(+)
6- Oxidase test:	negative
7-Swarming phenomena 	Swarming is described as the formation of concentric zones of bacterial growth able to cover the whole surface of the solid culture medium. Proteus species swarm in waves on nutrient agar, blood agar and chocolate agar covering the entire surface of the plate.

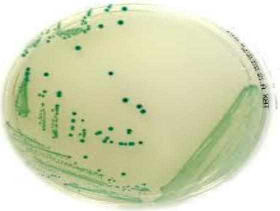
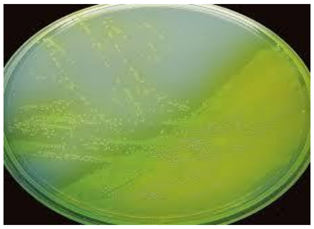
II- *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a common Gram-negative, aerobic (and at times facultative anaerobic), rod-shaped bacterium ,bacillus with unipolar motility that can cause disease in plants and animals, including humans. It has been identified as an opportunistic pathogen of both humans and plants. *P. aeruginosa* is the type species of the genus *Pseudomonas*.

In certain conditions, *P. aeruginosa* can secrete a variety of pigments, including pyocyanin (blue-green), pyoverdine (yellow-green and fluorescent), and pyorubin (red-brown). These can be used to identify the organism.

Laboratory Identification

Test	**Result**
1-Gram Stain:	Gram negative organism
2-McConkey's Medium	nonlactose fermenter
3-Urease production test	negative urease test.
4-IMViC: **Indole test:** **Methyl red test, Vogues-Proskaure’s test:** **Citrate utilization test:**	negative with Indole test. *P. aeruginosa* **is (MR-/VP-)** positive with citrate test.
5- Triple Sugar Iron Agar (TSI)	alkaline slant and butt, gas(-), H_2S(-)
6- Oxidase test:	oxidase positive
7-Motility agar: Many bacterial species are motile due to presence of Flagella **Motility**: Movement between two points.	**Brownian movement** : Vibrational movement due to bombardment with water molecules, not a form of motility **(Non-motile).**
8-Gelatin hydrolysis:	*positive*
9- Catalase test:	*positive catalase test*
10- Pyocyanin production **Pyocyanin (PCN)**	is a blue redox-active secondary metabolite that is produced by

	Pseudomonas aeruginosa.
11-Cetrimide agar (Alkyltrimethylammonium bromide) - **Cetrimide agar is a type of agar used for the selective isolation of the gram-negative bacteria, Pseudomonas.**	As the name suggests, it contains cetrimide, which is the selective agent against alternate microbial flora. Cetrimide also enhances the production of Pseudomonas pigments such as pyocyanin and fluorescein, which show a characteristic blue-green and yellow-green colour, respectively.  ***Pyocyanin pigment on cetrimide agar***  ***Fluorescein pigment on cetrimide agar***

Laboratory Report 6

1- Gram stain:………………………………

2- Shape:…………………………………….

3- Arrangement:……………………………

4- Color:…………………………………….

5- Confirmatory test:

NO:	Name	Result
1		
2		
3		
4		

6- Drawing:

7-Conclusion

The organism is:

Laboratory Report 7

1- Gram stain:………………………………

2- Shape:…………………………………….

3- Arrangement:……………………………

4- Color:…………………………………….

5- Confirmatory test:

NO:	Name	Result
1		
2		
3		
4		

6- Drawing:

7-Conclusion **The organism is:**

Laboratory Report 8

1- Gram stain:………………………………

2- Shape:…………………………………….

3- Arrangement:……………………………

4- Color:…………………………………….

5- Confirmatory test:

NO:	Name	Result
1		
2		
3		
4		

6- Drawing:

7-Conclusion **The organism is:**

Laboratory Report 9

1- Gram stain:………………………………

2- Shape:…………………………………….

3- Arrangement:……………………………

4- Color:…………………………………….

5- Confirmatory test:

NO:	Name	Result
1		
2		
3		
4		

6- Drawing:

7-Conclusion

The organism is:

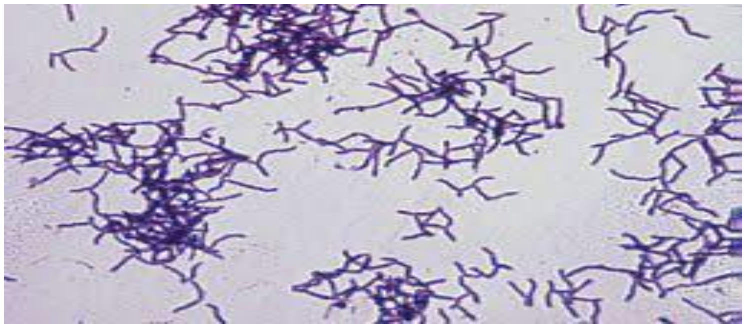
Module 3

DIAGNOSIS OF MICROBIAL INFECTION OF ACTINOMYCETES

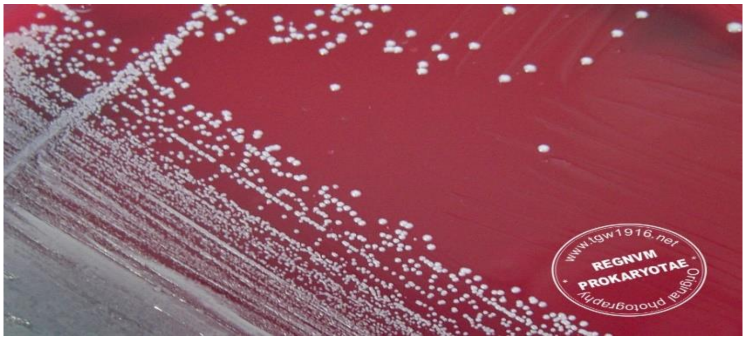
- Actinomycosis is a rare chronic disease caused by *Actinomyces* spp., anaerobic Gram-positive bacteria that normally colonize the human mouth and digestive and genital tracts
- Bacteria of the genus *Actinomyces* belong to the Actinobacteria phylum and Actinomycetales order and are related to other genera such as *Corynebacterium*, *Mycobacterium*, *Nocardia*, and *Propionibacterium*.
- More than 30 species of *Actinomyces* have been described. *Actinomyces israelii* is the most prevalent species isolated in human infections and is found in most clinical forms of actinomycosis.

Microbiological diagnosis of actinomycosis

1. A Gram stain: *Actinomyces* are non-spore-forming Gram-positive rods.

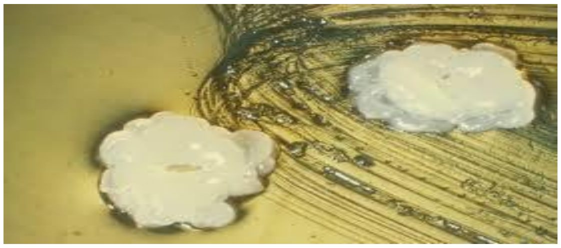


2. Growth of *Actinomyces:* is slow; incubation of at least 10 days is required before conclusion of a negative culture. Most *Actinomyces* spp. are facultative anaerobes.



- *Actinomyces* can be cultured on chocolate blood agar media at 37°C, brain heart infusion broth and Brucella Blood Agar with hemin and vitamin K1. The use of semi-selective media (such as phenylethyl alcohol or mupirocin-metronidazole blood agar) may increase isolation rates by inhibiting overgrowth of concomitant organisms.

3. colony morphology: *A. israelii* forms a "molar tooth" colony on agar and grows as clumps within broth



4. Biochemical profiling: Identification was classically based on phenotypic tests (urease, catalase, fermentation of sugars, etc) or on commercial

biochemical kits but, in fact, such tests can lead to misidentification of species and even of genus.

5. Serological assays
6. Molecular techniques such as

a) 16S rRNA sequencing serve as the reference for identification

b) Polymerase chain reaction PCR with specific primers can also be used for direct detection of *Actinomyces* in clinical material

c) Finally, matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) should be a quicker and accurate tool for *Actinomyces* identification in the future

Laboratory Report 10

1- Gram stain:………………………………

2- Shape:…………………………………….

3- Arrangement:……………………………

4- Color:…………………………………….

5- Confirmatory test:

NO:	Name	Result
1		
2		
3		
4		

6- Drawing:

7-Conclusion

The organism is:

Module 5

DIAGNOSIS OF MICROBIAL INFECTION OF FUNGI

- There has been an enormous increase in the frequency and severity of fungal infection in recent years.
- Diagnosis of invasive fungal disease (IFD) is challenging because

1. current diagnostic methods lack sensitivity and specificity,
2. or take too long to yield a result to be clinically useful.

Diagnosis:

1. Culture from a clinical sample is the gold standard for diagnosis of fungal infection
2. Direct microscopy
3. Histopathology
4. Serology: serologic tests for patient antibodies have been useful for non-culture-based diagnosis of fungal infection.

☐ Available technologies include immunodiffusion (ID), complement fixation (CF), and enzyme immunoassay (EIA).

4. Molecular diagnostics: polymerase chain reaction (PCR)

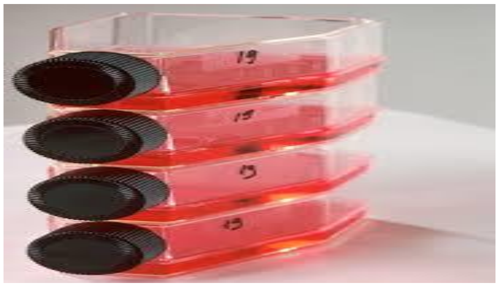
Module 6

DIAGNOSIS OF MICROBIAL INFECTION OF VIROLOGY

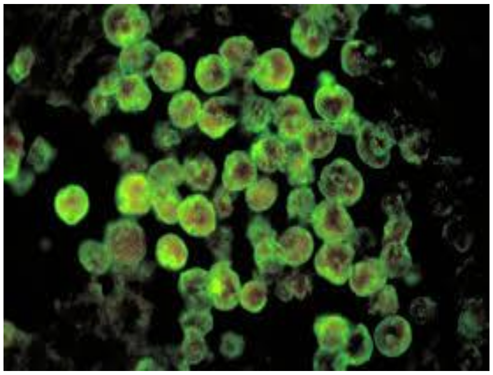
Methods Used in Diagnostic Virology

- Diagnostic virology has now entered the mainstream of medical practice. Multiple methods are used for the laboratory diagnosis of viral infections.

1. ***Cell culture**:* necessary because it is the only technique capable of providing a viable isolate that can be used for further characterization.



2. ***Nucleic acid detection**:* PCR analysis
3. ***Antigen detection**:* Methods of antigen detection include fluorescent antibody (FA) staining, immunoperoxidase staining, and EIA. Of these, FA staining is the most widely used in diagnostic virology.



4.

5. ***Serology**:* The diagnosis of viral infections by detection of specific antiviral antibodies is a traditional method whose clinical utility is limited by the need for comparison of acute and convalescent antibody titers.

Laboratory Report 11

1- Gram stain:………………………………

2- Shape:…………………………………….

3- Arrangement:……………………………

4- Color:…………………………………….

5- Confirmatory test:

NO:	Name	Result
1		
2		
3		
4		

6- Drawing:

7-Conclusion

The organism is:

Module 7

EXAMINATION OF CLINICAL SPECIMEN

Pathogens, particularly bacteria, coexist with harmless microorganisms on or in the human host. These pathogens must be properly identified as actual etiologic agents of infectious diseases. This is the purpose of clinical microbiology. The clinical microbiologist identifies agents and organisms based on:

1- Morphological
2- Microscopical
3- Biochemical
4- Immunological procedures
5- Molecular methods

Time is a significant factor in the identification process, especially in life-threatening situations.

The major concern of the clinical microbiologist is to isolate and identify microorganisms from clinical specimens rapidly. The purpose of the clinical microbiology laboratory is to provide the physician with information concerning the presence or absence of microorganisms that may be involved in the infectious disease process.

Clinical Specimens

In clinical microbiology, a clinical specimen defined as a portion or quantity of human material that is tested, examined, or studied to determine the presence or absence of particular microorganisms.

Characters of ideal clinical specimen:

1. The specimen selected should adequately represent the infectious disease.
2. A quantity of specimen adequate for complete examination should be obtained.

3. Attention must be given to specimen collection in order to avoid contamination from the many varieties of microorganisms indigenous to the skin and mucous membranes. Specimens may be collected by several methods using **aseptic technique**.

Aseptic technique refers to specific procedures used to prevent unwanted microorganisms from contaminating the clinical specimen. Each method is designed to ensure that only the proper material will be sent to the clinical laboratory.

4. The specimen should be forwarded promptly to the clinical laboratory.

5. If possible, the specimen should be obtained before antimicrobial agents have been administered to the patient.

Collection

1- The most common method used to collect specimens from the skin and mucous membranes (eye, ear, nose, throat, open wounds) is the **sterile swab**. **A sterile swab** is rayon- or dacron tipped polystyrene applicator. Many commercially manufactured swabs contain a transport medium designed to preserve a variety of microorganisms and to prevent multiplication of rapidly growing members of the population.

2- Specimens from blood and cerebrospinal fluid may be collected by **needle aspiration**. After the proper aseptic technique has been followed, a sample of fluid is drawn into a sterile tube that has been treated with an anticoagulant such as EDTA. The anticoagulant prevents the microorganisms from being entrapped in a clot, which would make isolation difficult.

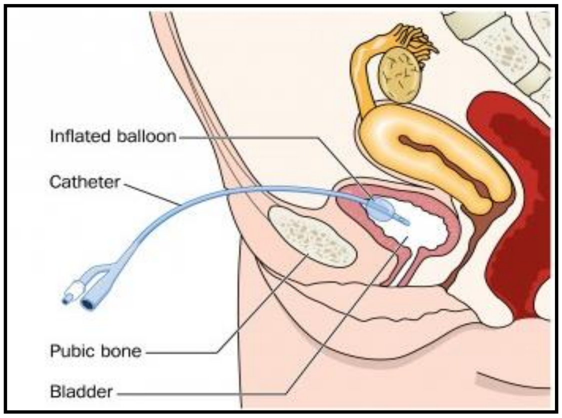
3-**Intubation** is the inserting of a tube into a body canal or hollow organ. For example, intubation can be used to collect specimens from the stomach. In this procedure, a long sterile tube is attached to a syringe and the tube is either swallowed by the patient or passed through a nostril into the person's stomach. Specimens are then withdrawn periodically into the sterile syringe.

4- Urine also may be obtained by the **clean-catch method**. After the patient has cleansed the urethral opening, a small container is used to collect the urine. In the clean-catch **midstream** method, the first urine voided is not collected because it will be contaminated with those microorganisms normally occurring in the lower portion of the urethra. Only the midstream portion is collected since it most likely will contain those microorganisms found in the urinary bladder.

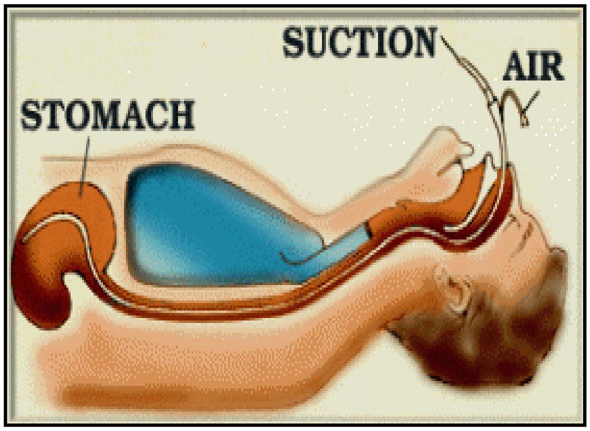
In case of urinary retention **catheters** are used (A catheter is a tubular instrument used for withdrawing or introducing fluids from or into the body). For example, urine specimens may be collected with catheters. Three types are commonly used. The hard catheter is used when the urethra is very narrow or has strictures. The French catheter is a soft tube used to obtain a single specimen sample. If multiple samples are required over a prolonged period, a Foley catheter is used.

5- Respiratory tract specimens are represented by the **sputum**. Specifically, sputum is the mucous secretion expectorated from the lungs, bronchi, and trachea through the mouth, in contrast to saliva, which is the secretion of the salivary glands. Sputum is collected in specially designed sputum cups.

Sample collection techniques seen in the following figure:


Inflated balloon
Catheter
Pubic bone
Bladder

Sputum cup


SUCTION
AIR
STOMACH

Handling:

Immediately after collection, the specimen must be properly labeled and handled. The person collecting the specimen is responsible for ensuring that the

1- Patient name

2- Age

3- Sex

4- Code number

5- Tests required for suspected pathogens

Transport:

Speed in transporting the specimen to the clinical laboratory after it has been obtained from the patient is of prime importance. Some laboratories refuse to accept specimens if they have been in transit too long.

Special treatment is required for specimens when the microorganism is thought to be anaerobic. The material is aspirated with a needle and syringe. It is practical to remove the needle, cap the syringe with its original seal, and bring the specimen directly to the clinical laboratory. Transport of these specimens should take no more than 10 minutes; otherwise, the specimen must be injected immediately into an anaerobic transport vial. Vials should contain a transport medium with an indicator, such as resazurin, to show that the interior of the vial is anaerobic at the time the specimen is introduced. Swabs for anaerobic culture are usually less satisfactory than aspirates or tissues, even if they are transported in an anaerobic vial.

Dealing with clinical samples after collection

Samples are transported to microbiological media which allow the growth of all suspected pathogens & culturing done on such media to obtain pure colonies of micro-organisms by streaking for isolation methodology.

Module 8

INTRODUCTION TO PARASITOLOGY

Parasitology: Is the science that deals with organisms (Parasite) that live on or within other organisms (Hosts).

The association between two organisms may be one of the following:

1. **Mutualism**: Mutual benefits is derived from the association.
2. **Symbiosis**: Mutual benefit, and the two organisms can't live independently.
3. **Commensalism**: One partner benefits (commensal) while the other (host) is unaffected. It may be called a non-pathogenic parasite.
4. **Parasitism**: One organism (parasite) lives at the expense of the other (host) which usually suffers from the association (pathogenic parasite).

Types of hosts

Final host or Definitive host: Harbors the adult or sexually mature parasite.

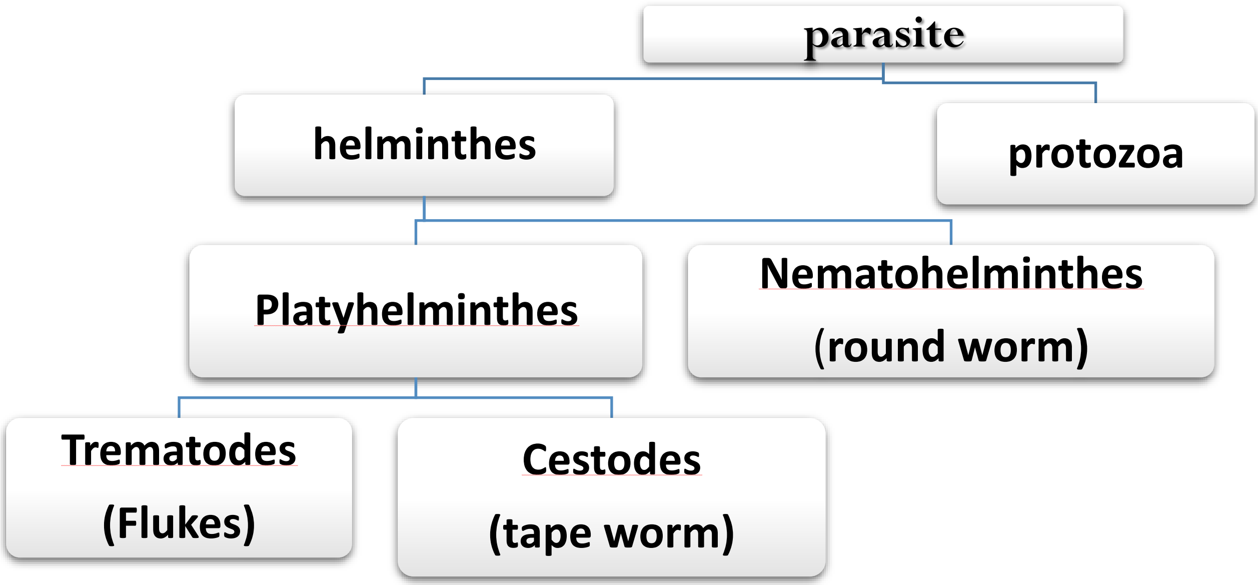
Reservoir host: Animals that harbor the parasites indefinitely with no ill effects.

Intermediate host: Harbors the immature or asexual stages of the parasite.

Vector: An arthropod that carries a parasite to its host.

Infective stage: the stage of parasite which cause disease.

Diagnostic stage: The stage that can be detected in the specimen.


parasite
helminthes
protozoa
Platyhelminthes
Nematohelminthes
(round worm)
Trematodes
(Flukes)
Cestodes
(tape worm)

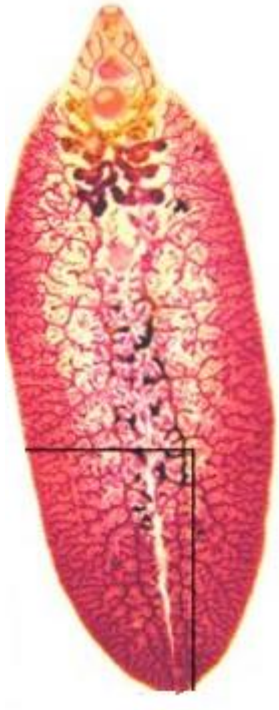
Module 9

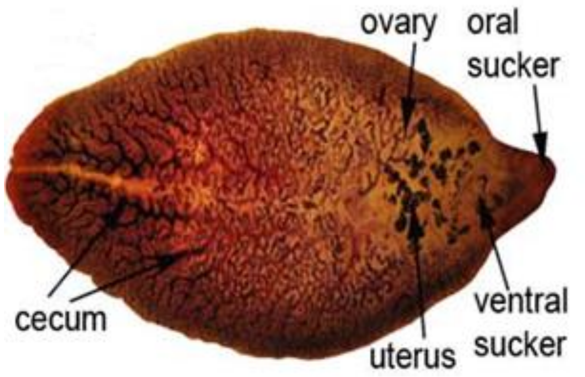
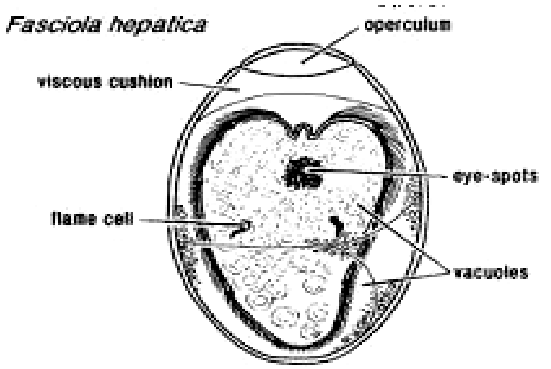
TREMATODA

Liver Flukes (FASCIOLAE)

1- *Fasciola gigantica*, adult :

- Length : 3-7 cm.
- Elongated with parallel borders.
- Ventral sucker is larger than oral one.
- Lateral compound and inner T- or Y-shaped caecal branches.
- 2 branched testes (in tandem).
- One branched ovary, anterior to the anterior testis, in the middle of the body.
- Numerous vitelline follicles at the lateral borders.



2- *Fasciola hepatica,* adult : a. Length : 2-3 cm. b. Shorter and broader flat worm with two converging lateral borders. c. Cephalic cone and shoulders are prominent anteriorly. d. Oral and ventral suckers are equal. e. The medial caecal branches are simple diverticula.	
3- Fasciola egg : - Oval. - Thin, operculated shell. - Size : 160X80 u. - Yellowish-brown. - Contains an immature embryo.	 Fasciola hepatica operculum viscous cushion eye-spots flame cell vacuoles

Definitive host - sheep, cattel(pig)and man.

Intermediate host - The snail Lymnea truncatula in

case of F. hepatica and L. cailaudi
in case of F. gigantica

Infective stage - Encysted metacercaria

Mode of transmission - Ingestion of aquatic plants or drinking water containing encysted metacercaria

Site of localization - Bile duct

Laboratory diagnosis:

1- **Microscopic examination:** for demnotration of operculated egg or the adult worm in stool specimen, duodenal or biliary aspirate.

2- **Serology:** using ELISA (Enzyme Linked Immunosorbent Assay) to detect antibodies in sera.

Activity

- **Drawing**

***F. gigantic*a adult worm**

***F. hepatica* adult worm**

Fasciola egg

Case study

A 36-year-old man suffering from intermittent fever, diarrhea, indigestion and abdominal pain in the right hypochondrium. Upon examination, he had a slightly enlarged tender liver and yellow colouration of the sclera. When questioned regarding his eating habits, the patient admitted to having a fondness for un-cooked water-cress and raw vegetables. An order was written for stool examination for ova and parasites. Blood sample was collected for complete blood count and liver function tests.

Haematology results showed evidence of anaemia and eosinophilia (60% eosinophils). The patient's liver enzyme levels were slightly elevated. The diagnosis was made microscopically after the observation of large, oval, 180X90 u, yellowish-brown, operculated eggs in the concentrated stool specimen.

QUESTIONS:

1. Which parasite might be causing this infection?

2. Where is this parasite found geographically?

3. Which other helminth lays eggs indistinguishable from the eggs described in this specimen?

4. How does transmission of this parasite occur?

5. What are the usual symptoms of the disease in humans?

6. How the diagnosis of this infection is usually made?

7. How do you exclude false diagnosis?

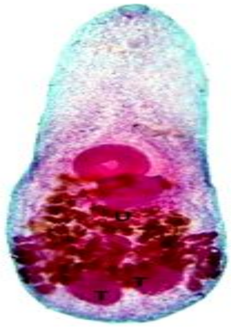
8. How this parasite is treated?

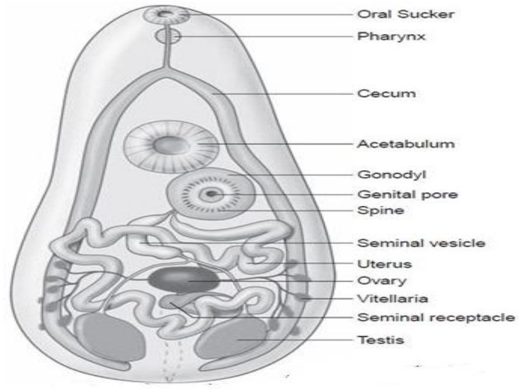
9. How can you control such infection?

1- Intestinal Trematodes (HETEROPHYES HETEROPHYES)

1- Adult :

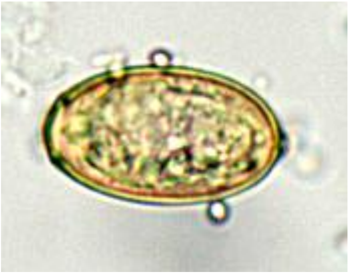
- Pyriform in shape with broad posterior end.
- Size : 1.5-3 mm.
- 3 suckers, oral (small),ventral (large) and genital.
- 2 simple intestinal caeca.
- 2 oval testes, opposite each other, posteriorly.
- Oval mid plane ovary in front of testes.
- Few large postero-lateral vitelline follicles.





2- Egg :

- Oval.
- Size : 30X15 u.
- Yellowish-brown.
- Thick-shelled.
- Operculated.
- Small knob posteriorly.
- Mature (contains miracidium).



Definitive host - Dog, cat and man.

Intermediate host - The snail Pirenella conica

Infective stage - Encysted metacercaria

Mode of transmission - Ingestion of raw fish containing encysted metacercaria

Site of localization - Small intestine

Laboratory diagnosis:

1- **Microscopic examination:** for demnotration of egg containing miracidia or the adult worm in stool specimen.

Activity

- **Drawing**

H. heterophyes adult worm

H. heterophyes egg

Case study:

The cases were 38-year old Korean worker who had been in Saudi Arabia for 4-6 years and returned home in 1983 or 1984 with gastrointestinal troubles. In Saudi Arabia they had eaten raw brackish water fishes such as the mullet. After the treatment with 10 mg/kg praziquantel and purgation with magnesium salt, a total of 19 specimens of *H.heterophyes* and 140 of *H. dispar* were collected. It is of interest that the worms persisted in a patient although he had been back in Korea for 14 months. This is the first report on imported heterophyiasis in Korea. Human infection by *H. dispar* is the first record in the literature.

1. Which parasite might be causing this infection?

2. How does transmission of this parasite occur?

3. What are the usual symptoms of the disease in humans?

4. How the diagnosis of this infection is usually made?

5. How this parasite is treated?

6. How can you control such infection?

3-HUMAN SCHISTOSOMES

1- *S. haematobium* Egg:

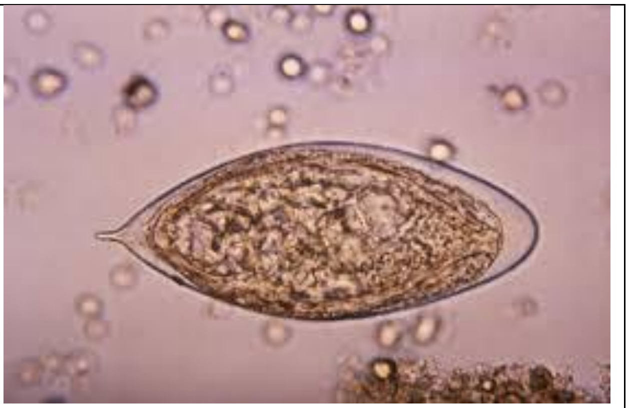
-Size: 120 x 60 µ.

-Shape: oval.

-Shell: thin with terminal spine.

-Colour: translucent.

-Contents: mature miracidium.



2- *S. mansoni* egg:

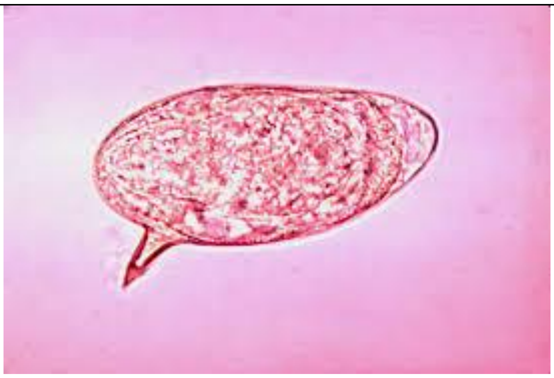
Size: 140 x 70 µ.

Shape: oval.

Shell: thin with lateral spine.

Colour: translucent.

Contents: mature miracidium.



Feature	***S. mansoni***	***S. Haematobium***

Geographic distribution	**Africa and America**	**Prevalent in Africa**
Site of localization	**Superior mesenteric veins draining the large intestine**	**Venous plexus of bladder**
Egg (diagnostic stage)	**Lateral spine**	**Terminal spine**
Intermediate Host	**Biomphalaria and Australorbis**	**Bulimus (Physopsis)**
Definitive Host	**Man**	**Man**
Infective stage	**Cercaria**	**Cercaria**

Laboratory diagnosis

1- **Microscopic examination** of eggs in stool (*S. Mansoni)* or in urin *(S. haematobium)*

Since eggs may be passed in small amounts their detection may enhanced by repeated examination or concentration procedures (formaline ethylacetate technique)

2- **Kato KatzTechnique**:
  - For enumeration of eggs
  - Most commonly used for evaluating epidemiology, effect of control measures, drug trials

3- Immunodiagnosis
  - Indirect hemagglutination using adult worm and egg antigens
  - Enzyme-Linked Immunosorbent Assay (ELISA) using soluble antigens of adults and eggs.

Activity

- **Drawing**

***S. haematobium* ova**

***S. Mansoni* ova**

Case study:

1) A 13-year-old male, from a village near Mansoura, presented to the Out-Patient Clinic of Mansoura University Hospital with complaints of painful urination, the presence of blood in his urine, fatigue, fever and general body aches. Upon examination, the physician ordered a urine analysis and urine culture to rule out a urinary tract infection.

Culture results were negative for pathogenic bacteria. Microscopic examination of the urine sediment revealed proteinuria, many RBCs (haematuria) and few white blood cells. Oval, translucent eggs with prominent terminal spines were also detected.

QUESTIONS:

1. Which parasite is the cause of this patient's infection?

2. How is this infection transmitted?

3. Mention the complications of this parasitic infection.

4. Describe the detected egg.

5. Compare this egg with those of other members of this genus.

6. Which types of specimens should be collected for diagnosis?

7. How is this infection diagnosed?

8. Describe the "hatching test" and mention its value.

9. What is the association of this infection with bladder cancer?

10. How is this infection treated?

11. How is infection with this parasite prevented and controlled?

2) A 22-year-old Egyptian woman was visiting American relatives and developed fever, malaise, dysentery and abdominal pain. Her relatives brought her to the family doctor for examination. Upon examination, she was noted to have liver tenderness. Blood was drawn for complete blood count and liver enzyme analysis. Three stool specimens were submitted for examination for ova and parasites.

The patient was noted to be mildly anaemic and had slightly elevated liverenzyme levels. Two of the 3 stool specimens revealed a small number of eggs. Each is oval, translucent, 140X70 p with a prominent lateral spine.

QUESTIONS:

1) Which parasite is causing this patient's infection?

2) How does this parasite differ from other human trematode parasites?

3) Give an account on Katayama syndrome?

4) How can we control such an infection?

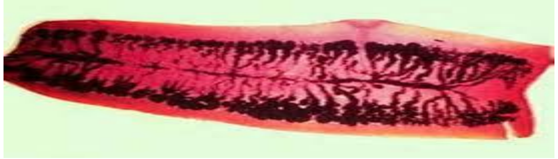
Module 10

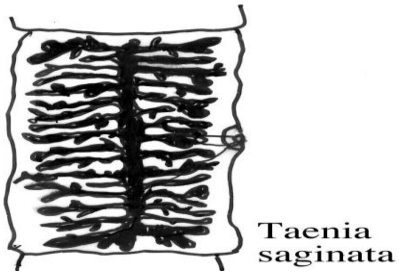
CESTODA

TAENIAE

1- *T. saginata* (beef tapworm) gravid segment :

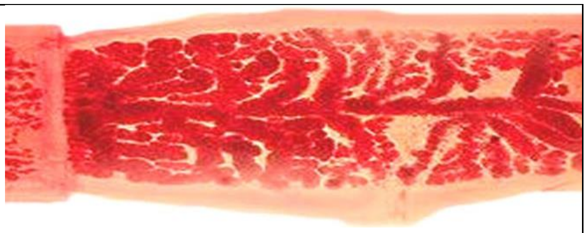
a- Longer than broad.
b- Size : 20X7 mm.
c- Main lateral uterine branches : 15-20 on each side.

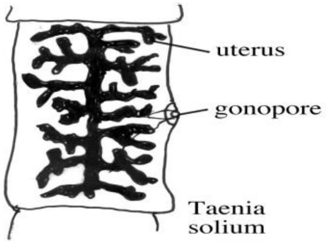




2- *T. solium* gravid (pork tapworm) segment :

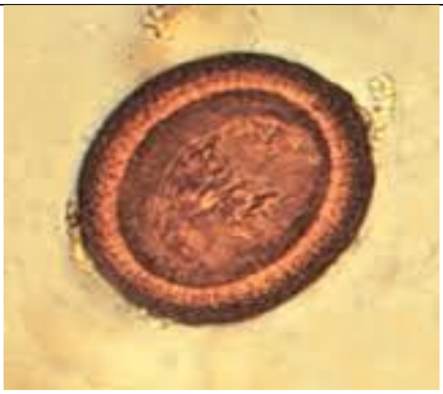
a- Longer than broad.
b- Size : 11X6 mm.
c- Main lateral uterine branches : 9-11 on each side.





3- *Taenia* egg :

a- Rounded.
b- 40 u. in diameter.
c- Brownish in colour.
d- With thick double-layer and radially-striated embryophore.
e- Contains hexacanth embryo.



	T. saginata	*T. solium*
Definitive host	- man.	- man
Intermediate host	- cattel	- pig
Reservoir host	- No reservoir host	- No reservoir host
Infective stage	- cysticercus bovis in beef	- cysticercus cellulosa
Mode of transmission	- Eating undercooked infected meat containing cysticercus bovis	- Eating undercooked infected pork meat containing cysticercus cellulosa
Diagnostic stage	- Terminal gravid segments and/ or eggs	- Terminal gravid segments and/ or eggs
Site of localization	- Small intestine	- Small intestine

Diagnosis

1- *T. saginata*

A. Clinically

Intestinal taeniasis caused by *T. saginata is suspected in endemic areas by the crawling movements of gravid segments in the peri-anal area.*

B. Laboratory Diagnosis

- Stool examination for the characteristic egg.
- Identification of the characteristic gravid segments in the stool. They are compressed between 2 slides and identification of lateral uterine branches.
- Peri-anal swabbing to detect *Taenia eggs in the perineum*

2- *T. solium*

- Stool examination for the characteristic eggs, gravid segmentsor scolex.
- Eggs can be differentiated from those of *T. saginata* by Ziel-Neelsen stain where eggs of *T. saginata* is acid fast (red) while T. solium is not acid fast.
- Identification of the characteristic gravid segments in the stool. They are compressed between 2 slides and identification and counting of lateral uterine branches can differentiate between the two types.

Activity

Drawing

***T. saginata* gravid segment**

***T. solium* gravid segment**

***Tanenia sp.* Ova**

Case study

A 29-year-old woman presented to the physician complaining of diarrhea, mild indigestion, hunger pains, loss of weight and frequent abdominal pain. She passed white segments, each about 2 cm long, with or without defecation causing perineal irritation and pruritus. She is fond of eating roasted meat.

The patient was instructed to submit three stool specimens, on alternate days that were examined for ova and parasites. Also, blood sample was drawn for complete blood count.

The blood count revealed eosinophilia (16% eosinophils). On examination of the concentrated stool sediments, several yellow-brown, spherical, 40 μ in diameter, thick-shelled eggs were detected. The eggs were characterized by radial striations. Gravid segments were also detected in the stool specimens; each contained 15-20 lateral uterine branches, on either side, when stained with an Indian ink.

QUESTIONS:

1. Which parasite is causing the patient's illness?

2. Name the 2 species of this genus which cause human disease.

3. Can they be differentiated by the morphological appearance of their eggs? Explain.

4. Can these 2 species be differentiated by the appearance of their proglottids? Explain.

5. Compare the morphological characteristics of the scolices of these 2 species.

6. What is the infective stage? What is the mode of infection?

7. Why is it important to differentiate between these 2 species?

8. How is this infection treated?

9. What is the sure sign of complete cure?

10. How can you control this parasite?

Module 11

NEMATODA

1- Intestinal nematode ASCARIS LUMBRICOIDES

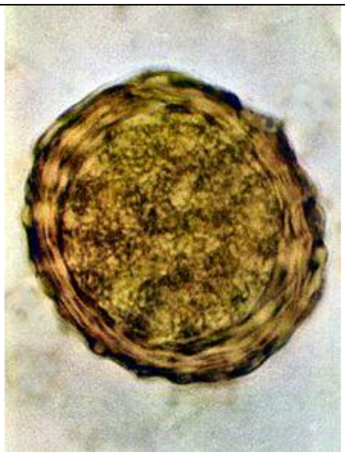
Fertilized egg :

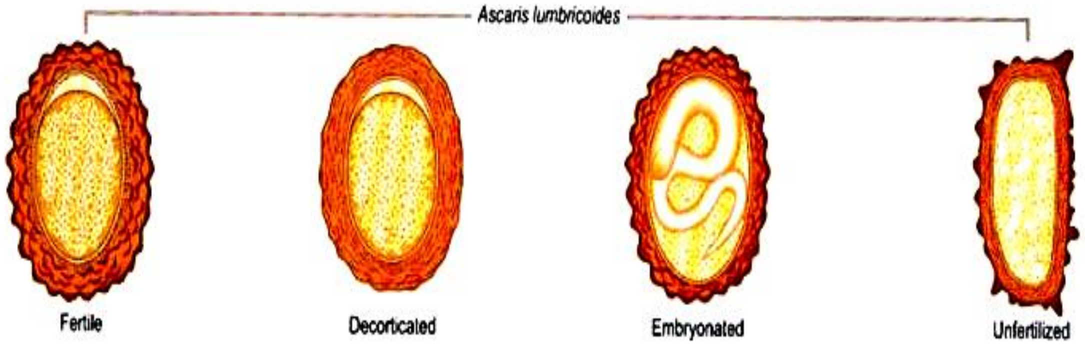
Broad oval.
Size : 60X45 u.

Yellowish-brown in colour.

Thick-shelled with regular albuminous mammillations.

Immature (contains large single cell stage embryo).





Definitive host - man.

Infective stage - egg containing a second stage rhabditiform larva

Mode of infection
- Ingestion of mature eggs containing 2nd stage rhabditiform larva, contaminating food (green raw vegetables), water or hands.
- Inhalation of mature eggs to the nasopharynx..

Diagnostic stage - Eggs

Site of localization - the adult worms live free in the small intestine (jejunum).

Stages in the life cycle: egg in the soil → larvated egg (with 2nd stage rhabditiform larva) ,→ ingested by man →larva →adult in the small intestine .

Diagnosis

- Clinical: symptom of intestinal ascariasis are indistinguishable from those of other intestinal helminthic infections.
- Laboratory
    1. Detection of eggs in stool. (direct smear, after concentration, Stoll's technique).
    2. Detection of migrating larvae in sputum or better in gastric lavage contents.
    3. Detection of adults passing out with or without stool or in vomitus.
    4. Eosinophilia (7 – 12%).
    5. Radiology.

Activity

- **Drawing**

Ascaris lumbricoides ova

Case study

A 21-year-old woman presented suffering from abdominal colic, nausea, vomiting and diarrhea. After a physical examination, which was un-remarkable, the physician ordered a stool analysis for eggs and parasites.

Microscopic examination of a concentrated wet-mount preparation revealed several types of eggs. These eggs had thick shells and were oval, with some being more broadly oval than others. Some eggs lacked the outer mammillated covering found on the majority of eggs.

The diagnosis of this intestinal parasitic infection was made on the basis of microscopic analysis of stool specimen.

QUESTIONS:

1. Which parasite would you suspect of causing this patient's infection?

2. Describe the variable appearance of eggs of this parasite.

3. Which nematodes are most likely to cause human intestinal infection?

4. What is the infective stage?

5. How is this infection transmitted?

6. Do you think that this patient can transmit this parasitic infection to other members of the family during food handling? Why?

7. Describe the clinical manifestations of this infection.

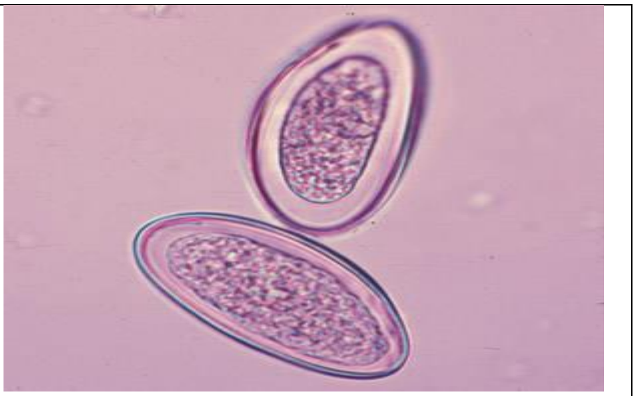
8. Which complications may cause this infection to be life-threatening?

9. How is this infection treated and controlled?

2-ENTEROBIUS VERMICULARIS

Egg :

a- D-shaped (plano-convex).
b- Size : 50X25 u.
c- Shell : double-walled.
d- Translucent.
e- Contains fully developed larva.



Definitive host	- Only man.
Infective stage	- fully embryonated eggs containing fully developed larvae
Mode of infection	- Ingestion of eggs through contaminated food and drink . - Autoinfection - Retro-infection - Air- borne infection - Contact with patients (Direct hand to hand or indirect contact by handling contaminated articles as clothes, bed linens)
Diagnostic stage	- Eggs
Site of localization	- Adult worm live in large intestine

Laboratory Diagnosis:.

- Microscopic identification of eggs collected in the perianal area is the method of choice for diagnosing enterobiasis. This must be done in the morning, before defecation and washing, by pressing transparent adhesive tape ("Scotch test", cellulose-tape slide test) on the perianal skin and then examining the tape placed on a slide.
- Alternatively, anal swabs or "Swube tubes" (a paddle coated with adhesive material) can also be used.

- Eggs can also be found, but less frequently, in the stool, and occasionally are encountered in the urine or vaginal smears. Adult worms are also diagnostic, when found in the perianal area, or during ano-rectal or vaginal examinations.

Activity

- **Drawing**

Enterobius vermicularis ova

Case study

A 7-year-old boy was not sleeping well, had been irritable, and had complained to his mother about anal itching and irritation. The boy's younger sibling also began to complain of similar symptoms.

The children were taken to the pediatrician for evaluation. He ordered a parasitological laboratory test to provide a strict diagnosis. A worm egg seen microscopically enabled the laboratory to identify the worm causing the symptoms.

QUESTIONS:

1. Which parasite is causing the children's discomfort?

2. Which type of laboratory procedure would the physician have ordered to make a diagnosis?

3. How is this procedure performed?

4. What are the precautions to be followed during performing this procedure?

5. How is the infection transmitted?

6. How is the diagnosis made, using this procedure?

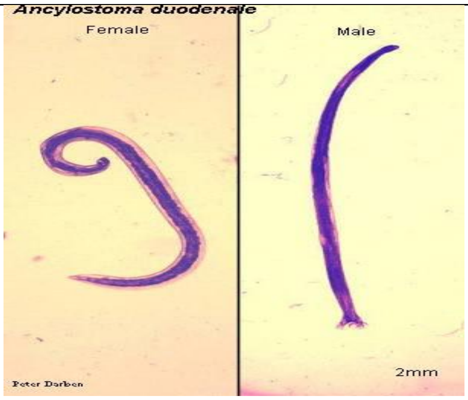
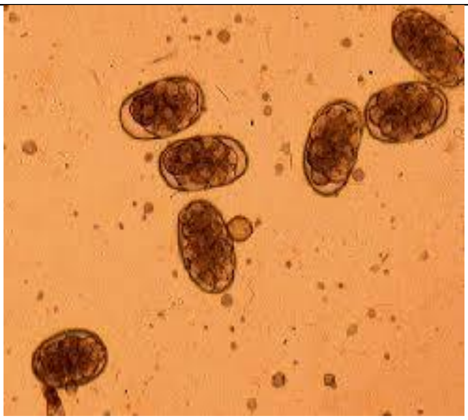
7. Which intestinal protozoon has been associated with this helminthic infection?

8. Should this patient be treated?

9. How is this infection treated?

10. What are the precautions to be followed to eradicate this parasitic infection?

ANCYLOSTOMA DUODENALE (hookworm)

Adult: adult are about 12 mm in length with the males smaller than females.	 Ancylostoma duodenale Female Male 2mm Peter Darben
egg: a- size: 60 X40 u. b- shape: Oval with blunt poles. Thin shell. c- Colour: translucent. d- Contents: immature ovum (4- cell stage). (empty narrow space between shell and contents)	

Definitive host - pigs, rodents and man.

Reservoir host - no reservoir host

Infective stage - infective filariform larva

Mode of infection

1) Man is infected when the infective filariform larva in the top layer of soil penetrates his intact skin
2) *Ancylostoma* infections may be also oral,

transmammary or transplacental.

Diagnostic stage - eggs.

Site of localization - adults live in the small intestine of man

Diagnosis:

1) Clinical diagnosis: the clinical picture is not sufficient to permit differentiation from other helminthic infection.

2) Laboratory diagnosis

-Stool examination for the characteristic ova or larvae in case of stored stool specimens for more than 24hours at room temperature.

Activity

Drawing

Ancylostoma duodenale larva

Case study

A 41-year-old farmer presented with vague gastrointestinal complains, fatigue, weakness, pallor and loss of weight. The physician ordered a stool analysis. Three stool specimens, collected on alternate days, were submitted for examination for ova and parasites. Blood sample was also drawn for complete blood count.

A moderate number of eggs were detected with occult blood in the stool. Each is oval, translucent with blunt poles and clear extra-embryonic space. A single larva was also observed in one stool specimen. This specimen had sat overnight at room temperature before being examined. Hematology result revealed hemoglobin of 10.0 gm/dl.

QUESTIONS:

1. Based on the patient's symptoms and morphology of the detected eggs, which parasites are possible causes of the patient's symptoms?

2. Compare the geographical distribution of these 2 parasites.

3. How is this infection transmitted to humans?

4. Would you expect to find both eggs and larvae of these parasites in an infected patient's stool specimen? Explain.

5. Describe the 2 larval stages of these helminths.

6. Which other nematode has larval stages, in the stool that may be confused with the larvae of these parasites?

7. What are the causes and type of anaemia that may occur in children heavily infected with these parasites?

8. How is this infection treated?

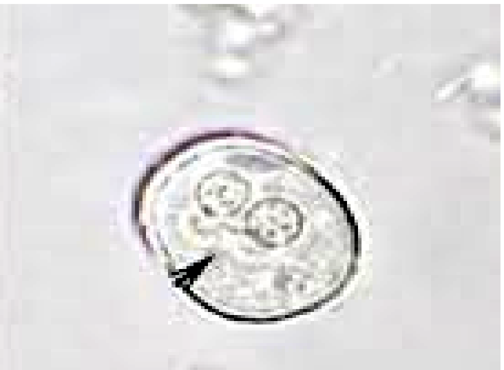
9. How can you control this parasitic infection?

Module 12

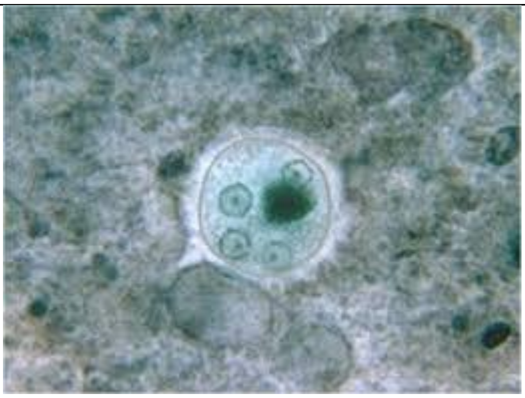
PROTOZOA

1-Sarcodina

1- Entamoeba coli

Cyst: a- Rounded. b- 15-25 U. in diameter. c- Contains 8 nuclei (at different levels). d- No chromatoid bodies in mature cyst.	

2- Entamoeba histolytica

Cyst : a- Rounded. b- 10-20 μ in diameter. c- Contains 4 nuclei (at different levels) and rod-shaped chromatoid bodies with rounded ends.	

Definitive host - man.

Reservoir host - dogs, rat and monkey

Infective stage - four nucleated cyst

Mode of infection ☒ ingestion of mature cysts in fecally contaminated food, water, or hands

Diagnostic stage - cyst and trophozoite

Site of localization - lumen of the colon

Diagnosis

- Symptoms, history and epidemiology are the keys to diagnosis.
- In the laboratory, the infection is confirmed by finding cysts in the stool.

Activity

- **Drawing**

Entamoeba histolytica cyst

Case study

A 22-year-old male college student visited his family doctor complaining of crampy abdominal pain, malaise, nausea, fever and bloody mucoid diarrhea. He had been passing 6-8 loose stools daily for 6 days.

Stool specimens were collected on 3 alternate days and sent to the laboratory.

The stool culture was negative for pathogenic bacteria. No parasitic ova were detected in the concentrated sediment of the specimens. A small number of amoeboid trophozoites, measuring 15-25 p in diameter containing single nucleus and few R.B.Cs. in the finely granular cytoplasm were seen. No cyst forms were observed. By the permanent trichrome stain, the nucleus has a central karyosome with fine, regularly distributed peripheral chromatin granules on the inner surface of the nuclear membrane.

QUESTIONS:

1. Based on the patient's symptoms, which intestinal parasitic infection do you think he has?
2. What would you suspect the consistency of this patient's stool to be? Why?
3. How is this protozoon transmitted?
4. Discuss the pathogenicity of amoebiasis.
5. Describe the clinical manifestations of this infection.

6. Describe the ulcers formed in sever cases of amoebic dysentery.

7. What is the cause of diarrhea in intestinal amoebiasis?

8. How do the symptoms of amoebic dysentery mimic those of bacillary dysentery?

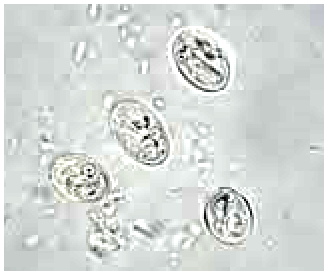
9. Is this parasite capable of causing extra-intestinal infection? Explain.

10. How should this patient be treated?

11. Discuss the methods of prevention and control of this infection.

2-Mastigophora

INTESTINAL FLAGELLATES Giardia intestinalis

Cyst: a- Oval. b- Size: 10X5 u. c- Double-walled. a- 4 nuclei at one pole. b- Remnants of axostyles and parabasal body.	

Definitive host - man.

Reservoir host - dogs, rodents, birds, sheep, cattle and pigs

Infective stage - four nucleated cyst

Mode of infection ☒ ingestion of mature cysts in fecally contaminated food, water, or hands

Diagnostic stage - cyst and trophozoite

Site of localization - duodenum, upper jejunum, bile ducts andgall bladder

Diagnosis

- Symptoms, history, epidemiology are used in diagnosis. *Giardia* caused dysentery is distinct from other dysenteries due to lack of mucus and blood in the stool, lack of increased PMN leukocytes in the stool and lack of high fever.
- Cysts in the stool and trophs in the duodenum can be identified microscopically after content has been obtained using a string device (Enterotest®).

Activity

- **Drawing**

Giardia intestinalis cyst

Case study

A 19-year-old woman presented to the physician suffering from profuse watery diarrhea, crampyepigastric pain and foul-smelling flatulence. The patient was instructed to submit a stool specimen for routine examination.

Three stool specimens were collected, on alternate days, and submitted for examination for ova and parasites. A wet mount and permanent trichrome stain microscopic examination revealed a small number of oval trophozoites, measuring 12X6 p. The structure of this trophozoite gave the overall appearance of "smiling face". Some cysts, having 4 nuclei, the characteristic median bodies and longitudinal fibers were also seen.

QUESTIONS:

1. Which protozoan parasite is causing this infection?

2. Which form of this parasite is infectious? Draw it.

3. How does the structure of these trophozoites account for the "smiling face”?

4. Name the habitat of this parasite.

5. How is this parasite transmitted?

6. How does this parasite attach itself to the intestinal wall?

7. Which condition might result as a sequence of this attachment?

8. Describe the pathogenicity of this protozoon.

9. How is the laboratory diagnosis of this infection made?

10. What is the value of the duodenal aspirate in the diagnosis of this infection?

11. How is this infection treated?

12. How can this infection be prevented and controlled?

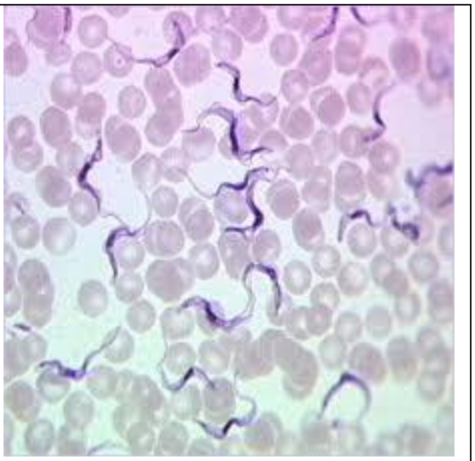
2-HAEMO-SOMATIC FLAGELLATES

Polymorphic trypanosomes

(T. gambiense, T. rhodesiense):

a- Elongated bodies and multi-sized (20-30 u. in length).
b- Extra-cellular.
c- Central vesicular nucleus.
d- Posterior kinetoplast.
e- Long undulating membrane.
f- Free flagellum.

Multiple in the blood film.



Definitive host - man.
Reservoir host - Tsetse fly
Infective stage - Metacyclic trypomastigotes
Mode of infection - Mechanical or direct transmission
- Cyclical transmission
Diagnostic stage - trypomastigotes
Site of localization - blood, lymph, lymph nodes, spleen, or cerebrospinal fluid

Diagnosis

- Detection of parasite in the bloodstream, lymph secretions and enlarged lymph node aspirate provides a definitive diagnosis in early (acute) stages.
- The parasite in blood can be concentrated by centrifugation or by the use of anionic support media.

- Cerebrospinal fluid must always be examined for organisms.
- Immuno-serology (enzyme-linked immune assay, immunofluorescence) may be indicative but does not provide definite diagnosis.

Activity

- **Drawing**

Trypanosomes

Case study:

A 37-year-old man was admitted to the hospital for fever and an inflamed foot. He had spent the previous three months touring South America, southern and eastern Africa, and Kathmandu. Ten days before admission, he visited game reserves in Tanzania, where he reported multiple tsetse fly bites. Six days later, he developed pain in the left foot and high fever. The next day, he noted progressive swelling on the dorsum of the foot and he was evaluated at a clinic in Katmandu. A malaria smear was negative and he was prescribed cephalexin. He continued to have intermittent high fever (to 41°C [105.8°F]) and developed vomiting, headache, myalgia, and a diffuse rash. A repeat malaria smear was negative, but his white cell count was 4000 per mm3 with 10% band forms. He returned to the United States and was admitted to the hospital. A shallow ulceration was present on his left instep, encircled by bullae at the margin and surrounded by purple discoloration. The foot lesion was 5 cm in diameter. The following images show what was observed on a thin film made from EDTA blood.

1. Which protozoan parasite is causing this infection?

2. Which form of this parasite is infectious? Draw it.

3. Name the habitat of this parasite.

4. How is this parasite transmitted?

5. Which condition might result as a sequence of this infection?

6. Describe the pathogenicity of this protozoon.

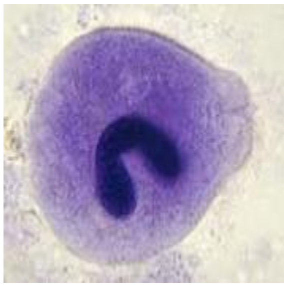
7. How is the laboratory diagnosis of this infection made?

8. How is this infection treated?

9. How can this infection be prevented and controlled?

Module 13

3-CILIATA *(Balantidium coli)*

Trophozoite : a- Oval in shape. b- 100X50 u. c- Covered with cilia. d- Anterior cytostome. e- 2 nuclei : i- Macronucleus (kidney- shaped). ii- Micronucleus (rounded). f- 2 posterior contractile vacuoles	

Definitive host - man.

Infective stage - cyst

Mode of infection ☒ ingestion of cysts in fecal contaminated food or water of farm animals

Diagnostic stage - cyst and trophozoites

Site of localization - large intestine

Diagnosis

- Cysts-formed stools
- Active trophs-diarrheic stools.

Activity

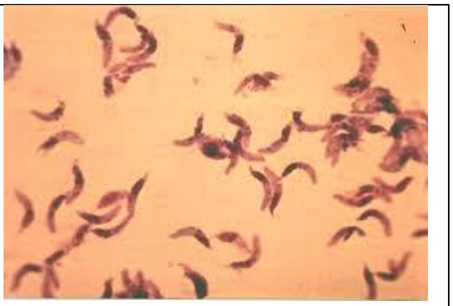
- **Drawing**

Balantidium coli trophozoite

Module 14

COCCIDIA

Toxoplasma gondii

Trophozoite: a- Crescent-shaped. b- One pole is more rounded than the other. c- 6X3 u. d- Nucleus is eccentric (near the rounded end). e- Para-nuclear body (near the pointed end).	

Definitive host	- domestic cats and their relatives.
intermediate host	- birds, rodents, wild game, animals bred for human consumption
Infective stage	- oocyst
Mode of infection	- ingesting food or water contaminated with cat feces or other feces-contaminated materials (eg, soil) or contact with a pet cat's litter. - ingesting undercooked meat containing tissue cysts. - Rarely, human infection results from blood transfusion or organ transplantation. - Rarely, transplacental transmission from mother to fetus occurs

Diagnostic stage - tachyzoites

Site of localization - skeletal muscle

Diagnosis

- Serologic testing
- PCR

Activity

- **Drawing**

Toxoplasma gondii

Case study:

A 33-year-old man with AIDS had been suffering from headache, fever and fatigue for several weeks. He also had a history of disorientation, confusion and convulsive attacks.

Serological tests for Toxoplasma-specific immunoglobulin M were negative. A computed tomography (CT) revealed multiple cerebral lesions. After being treated with a combination of pyrimethamine and sulfonamide, the patient appeared to recover from the infection.

QUESTIONS:

1. What is the likely diagnosis of this patient's parasitic illness?

2. What is the association between the patient's history of AIDS and his infection?

3. Which other group of individuals is at risk when infected with this parasite?

4. How is this infection transmitted?

5. Describe the infective sage(s), to man, of this protozoon.

6. Why was serology negative for this patient?

7. How is the diagnosis of this infection made?

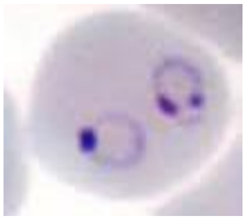
8. How can this infection be prevented and controlled?

Module 15

SPOROZOA *(Plasmodium malariae)*

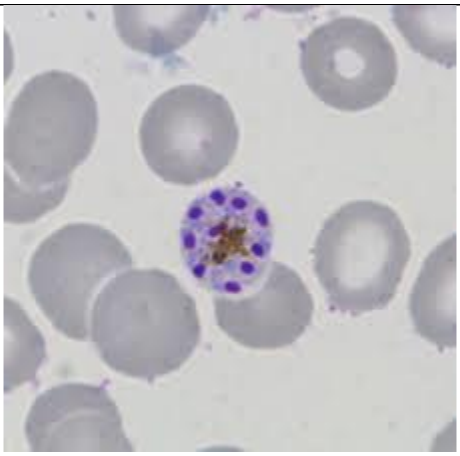
1- Ring form:

a- Single ring.
b- Large (fills 1/2 R.B.C.).
c- Enlarged parasitized RBCs.



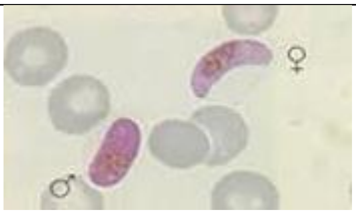
2- Schizont:

a- Large (fills R.B.C.).
b- Contains 8 (6-12) merozoites arranged regularly (rosette-shape).
c- Normal size of parasitized RBCs.



3- Gametocyte :

a- Large (fills R.B.C.).
b- Rounded or oval.



Definitive host - man.

Reservoir host - female Anopheles mosquito

Infective stage - sporozoites

Mode of infection - Mechanical or direct transmission

Diagnostic stage - Ring stage, mature trophozoite, schizont and gametocytes

Site of localization - Blood and liver

Diagnosis

- **Clinical Diagnosis** :is based on the patient's symptoms and on physical findings at examination
- **Microscopic Diagnosis:** by examining a drop of the patient's blood, spread out as a "blood smear" on a microscope slide. Prior to examination, the specimen is stained (most often with the Giemsa stain) to give the parasites a distinctive appearance.
- **Antigen Detection** use a dipstick or cassette format, and provide results in 2-15 minutes.
- **Molecular Diagnosis** using polymerase chain reaction (PCR).
- **Serology** detects antibodies against malaria parasites, using either indirect immunofluorescence (IFA) or enzyme-linked immunosorbent assay (ELISA).

Activity

- **Drawing**

Ring stage

schizont

Gametocytes

Case study

A 39-year-old man resented to the physician suffering from persistent night sweats, headache, intermittent fever and chills, which occurred approximately every 48 h. Clinical examination revealed high temperature (39.6° C), low pulse rate (65/min.), soft palpable spleen and tinge of jaundice. Blood was drawn for laboratory studies including complete blood count, thin and thick smears for parasites.

The patient was slightly anaemic, with a hemoglobin level of 9.5 gm/dl. Examination of Giemsa-stained blood films revealed the presence of an enlarged R.B.Cs. containing trophozoite stages. Several irregular amoeboid trophozoites containing brown granules were seen. Eosinophilicstibbling was visible in the cytoplasm of the RBCs. A few round to oval gametocytes were seen. Based on these findings, a diagnosis of infection with a blood parasite was made.

QUESTIONS:

1. Which parasite is infecting this patient?

2. Which disease does this patient have?

3. Describe the morphological characteristics of the parasitic stages which may be detected in thin Giemsa-stained smears.

4. Name the species and the types of diseases caused by these species.

5. Comment on the size of parasitized R.B.Cs. in infection with the different species of this genus.

6. Describe the clinical manifestations caused by this parasite.

7. Which serious complications may occur with this infection?

8. When should blood be collected, when this infection is suspected?

10. Why should thin and thick blood films be ordered to diagnose this disease?

11. In addition to thin and thick blood smears, which other laboratory tests are available for to diagnose this infection?

12. How would this patient be treated?

13. Differentiate between the types of recurrence (relapse and recrudescence) of this disease. What are the causes of each?